Design, Synthesis and Antimalarial Evaluation of Novel Azafluorenone Analogues

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Design, Synthesis and Antimalarial Evaluation of Novel Azafluorenone Analogues

By

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DECLARATION

This thesis entitled “Design, synthesis and antimalarial evaluation of novel azafluorenone analogues” is based on the work conducted by the author in Institute of Pharmaceutical Science at King’s College London between October 2011 and September 2014. All of the work described herein is original unless otherwise acknowledged in the text or by references. None of the work has been submitted for another degree at this or any other university.

Xiangchen Huang

ABSTRACT

With the rapid spread of drug resistance to chloroquine, the development of effective, safe, and affordable antimalarial drugs has become one of the most pressing health priorities worldwide. Two azafluorenone alkaloids isolated from *Mitrephora diversifolia* have been proved to possess antimalarial activity against chloroquine-resistant *Plasmodium falciparum*. To explore the structure-activity relationship of the azafluorenone family, twenty four analogues with various structural modifications have been prepared *via* a concise synthetic route utilising Suzuki cross-coupling reactions and intramolecular Friedel-Crafts acylations as the key steps. A series of hybrid molecules conjugating the azafluorenone core and the amino side chains present in 4-aminoquinoline antimalarial agents have also been synthesised. Fourteen of the azafluorenones have been evaluated for antiplasmodial activity by [^{3}H]hypoxanthine-incorporation assay. Eight azafluorenones display activity against chloroquine-sensitive *Plasmodium falciparum* 3D7, and nine azafluorenones are active against chloroquine-resistant *Plasmodium falciparum* K1. The hybrid molecules prepared possess a much higher antimalarial potency than the other azafluorenones, which can be attributed to the additional interaction between the amino side chains and the target site. An *in vitro* β-haematin inhibition assay has been conducted to probe the mode of action of the azafluorenones. However, only two azafluorenones show mild inhibitory activity against β-haematin formation. Four distinct synthetic routes have been investigated for the total synthesis of the parent antimalarial natural product. However, each of these fails to afford the desired alkaloid.

An efficient one-pot method for the synthesis of pyrido[3,2-*c*]coumarins has been developed. Twenty seven pyrido[3,2-*c*]coumarin derivatives have been prepared in moderate to excellent yields under the optimised thermal conditions, confirming the versatility of this protocol. Nine pyrido[3,2-*c*]coumarin analogues have been synthesised in moderate to good yields under microwave-assisted conditions. The mechanism of this one-pot reaction has been explored. The good yields, concise synthetic route, wide availability of substrates and milder reaction conditions render this one-pot protocol superior to other commonly used methods and make it a feasible approach to the synthesis of pyrido[3,2-*c*]coumarins in gram quantities.

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ABBREVIATIONS

Ac	acetyl
Ar	aryl
AQ	amodiaquine
Bu	butyl
DAMPA	2,4-diamino-N^{10}-methylpteroic acid
DCM	dichloromethane
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DHFR	dihydrofolate reductase
DHPS	dihydropteroate synthase
DMAP	4-dimethylaminopyridine
DME	dimethoxyethane
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulfoxide
equiv	equivalent(s)
ESI	electrospray ionisation
Et	ethyl
HPLC	high performance liquid chromatography
HsHsp90	human heat shock protein 90
Hsp	heat shock protein
HTS	high-throughput screening
IR	infrared
IC_{50}	half maximal inhibitory concentration
Me	methyl
Ms	methanesulfonyl
NMR	nuclear magnetic resonance
$PdCl_2$(dppf)	1,1'-bis(diphenylphosphino)ferrocene-palladium dichloride

PfCRT	*Plasmodium falciparum* chloroquine resistance transporter
PfHsp90	*Plasmodium falciparum* heat shock protein 90
PG	protecting group
Ph	phenyl
PPA	polyphosphoric acid
ppm	parts per million
Pr	propyl
Ts	toluenesulfonyl
Py	pyridine
QN	quinine
SDS	sodium dodecyl sulphate
TBAF	tetrabutylammonium fluoride
TBDMS	*tert*-butyldimethylsilyl
Tf_2O	trifluoromethanesulfonic anhydride
THF	tetrahydrofuran
TLC	thin layer chromatography
TMEDA	tetramethylethylenediamine

CHAPTER 1

Design, Synthesis and Biological Evaluation of Azafluorenone Analogues

1.1 Introduction

Malaria, a tropical disease caused by unicellular protozoan parasites of the genus *Plasmodium*, has remained a global public health concern for centuries. Currently, approximately 40% of the world's population lives in regions with a high risk of malaria (Figure 1.1).[1] Malaria is transmitted *via* the bite of a female *anopheles* mosquito. The parasites infect and destroy red blood cells where they multiply rapidly giving rise to fever, severe anaemia, coma, and even death if untreated. There are five *Plasmodium* species infecting humans, which can be distinguished by morphology, *Plasmodium vivax*, *Plasmodium falciparum*, *Plasmodium ovale*, *Plasmodium malariae* and *Plasmodium knowlesi* which was recently identified.[2] *Plasmodium falciparum*, the most prevalent species across the globe, can cause the infection of erythrocytes in the vessels of brain, giving rise to fatal cerebral malaria.[3] According to *World Malaria Report 2014* by World Health Organisation, there were approximately 198 million documented cases of malaria and an estimated 584 000 deaths in 2013. The actual number of deaths may be significantly higher, as the precise data is not available in many rural areas, and many cases are undocumented in these areas. Moreover, since international travel has become commonplace, malaria is no longer confined to the tropical regions and imported malaria has become an increasing public health issue across the world.

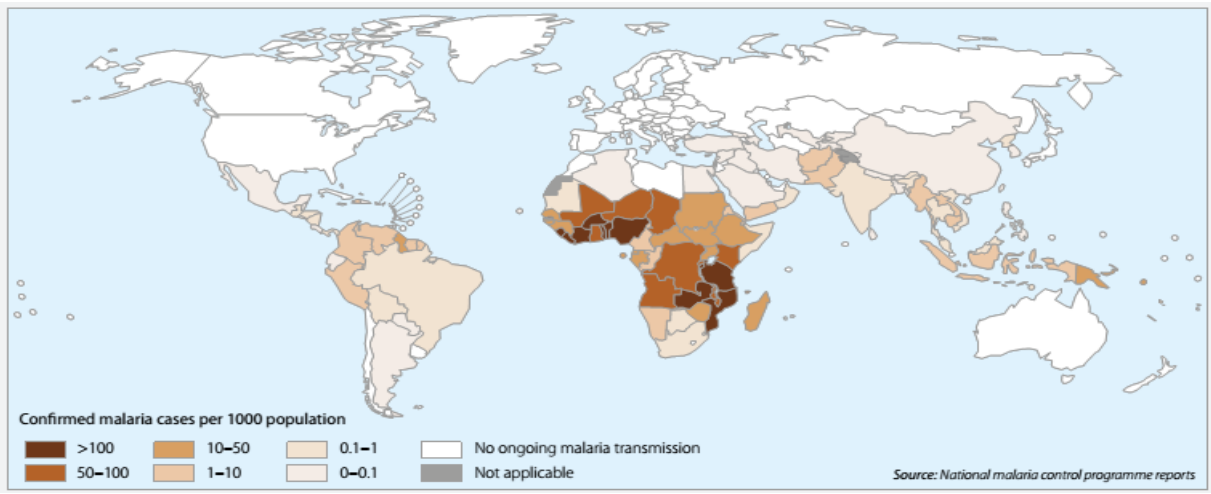


Figure 1.1 Countries with ongoing transmission of malaria, 2013.[4]

The most intractable problem for the control and prevention of malaria currently is the wide spread of resistance to the mainstay antimalarial chloroquine.[3] In addition, the recent emergence of resistance towards the antimalarial drug artemisinin in southeast Asia also poses a serious threat to the global control of malaria.[4] Owing to the rapid spread of drug resistance, the development of effective, safe, and affordable drugs for the treatment of malaria remains one of the most pressing health priorities worldwide.

1.1.1 Life Cycle of Malaria Parasites and Clinical Symptoms of Malaria

The infectious stages of the malaria parasite reside in the salivary glands of female *anopheles* mosquitoes that bite humans. As shown in Figure 1.2, the sporozoite form of the parasite is transferred into the blood stream during blood extraction of mosquitoes. Sporozoites rapidly enter the liver cells, where they conceal themselves from the host's immune system.[3] These sporozoites turn into hepatic schizonts, encompassing a tremendous number of merozoites. The rupture of mature schizonts gives rise to the release of numerous merozoites into the blood stream, initiating the erythrocytic phase of the parasite's life cycle. Alternatively, by developing into dormant hypnozoites, some sporozoites of *Plasmodium vivax* and *Plasmodium ovale* will remain in liver cells and result in the reactivation and relapse months or even years after the initial infection. In contrast, *Plasmodium falciparum* and *Plasmodium malariae* lack this liver-persistent phase. The merozoites entering the blood stream rapidly invade red blood cells, where they fuel their activity by consuming host's haemoglobin. In the erythrocyte, parasites develop from the ring stage *via* the trophozoite stage into the blood schizont, leading to the burst of erythrocyte and the release of a number of new merozoites into the blood stream as well. The newly released merozoites invade other red blood cells, commencing a new erythrocytic cycle (Figure 1.2). This process, from invasion of the erythrocytes to the rupture of the blood schizont, is referred to as the asexual life cycle, whilst the clinical signs and symptoms of malaria emerge due to the release of parasites' waste and cell debris.[3]

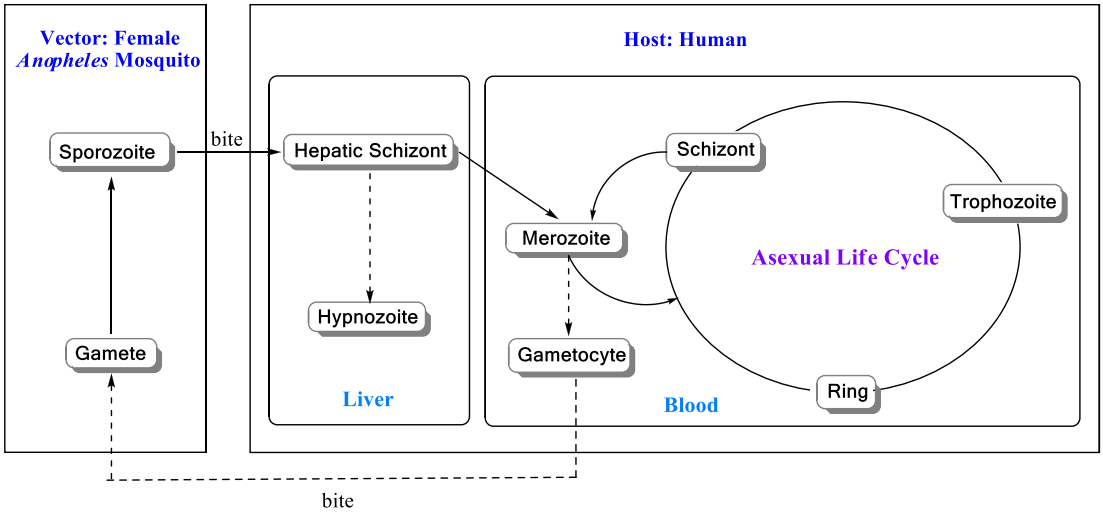


Figure 1.2 Life cycle of malaria parasites.

After a number of asexual life cycles, some merozoites evolve into gametocytes, the sexual form of parasites ready for the migration to an *Anopheles* through a subsequent blood meal. These gametocytes undergo sexual reproduction in the gut of *Anopheles* and are transferred to the salivary gland, awaiting the commencement of a new infection cycle (Figure 1.2).[3]

The clinical symptoms of malaria include fever, chill, headache, abdominal and back pain, nausea, diarrhoea and vomiting. Although the symptoms of *Plasmodium vivax*, *Plasmodium ovale*, and *Plasmodium malariae* infections can be sometimes severe in patients, seldom do these parasites induce fatal diseases. In contrast, *Plasmodium falciparum* malaria can develop from uncomplicated to severe malaria within a few days, giving a fatal outcome in 10-40% of the severe malaria cases.[5]

1.1.2 Established Antimalarial Drugs

Currently used antimalarial agents stem from five major drug classes. In most cases, antimalarials target against the asexual erythrocytic stage of the parasite. The main classes of blood schizontocides encompass 4-aminoquinolines, arylamino-alcohols, and

antifolate agents. The natural endoperoxide artemisinin and its semisynthetic derivatives as well as the synthetic analogues represent the newer antimalarial drugs. Contrasting with the other antimalarials, 8-aminoquinolines are active against both the pre-erythrocytic liver stage and the sexual gametocyte stage.[5]

1.1.2.1 4-Aminoquinolines

4-Aminoquinolines are the main family of antimalarial drugs used in the treatment and prophylaxis of malaria.[3] These drugs can be readily synthesised and are of low cost. The widely accepted theory regarding the mode of action of 4-aminoquinolines is related to their inhibition of haemozoin formation.[6] In the erythrocytic cycle, malaria parasites acquire essential amino acids as nutrients for the growth and proliferation by degrading the haemoglobin of the host red blood cell. Digestion of this food source takes place in the acidic compartment known as the digestive vacuole, which is a lysosome-type organelle with a pH value of approximately 5. During the degradation of haemoglobin, the toxic and soluble haems are generated as a by-product. The parasite disposes of these free haems by crystalising them into an insoluble and nontoxic polymer haemozoin to achieve the detoxification. The 4-aminoquinoline in the digestive vacuole of a parasite forms a quinoline-haem complex, which adsorbs onto the actively growing face of haemozoin, capping the haemozoin crystal and blocking the growth of the haemozoin polymer (Figure 1.3).[6] As a consequence, a great number of toxic free haems are released, resulting in the death of the parasite.

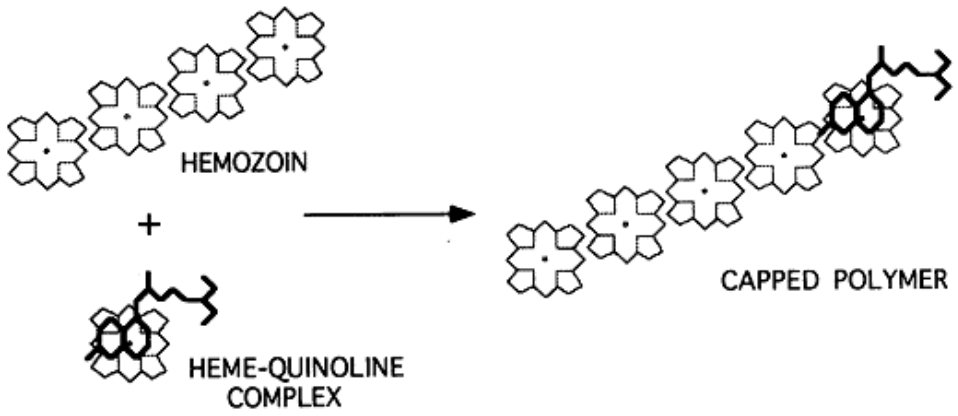
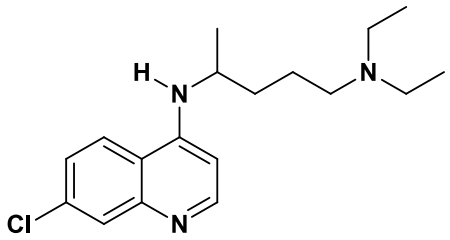


Figure 1.3 Model of inhibition of haemozoin formation by 4-aminoquinolines.[6]

Chloroquine **1** (Figure 1.4), introduced in the 1940s, has been the mainstay of therapy and prophylaxis of malaria for forty years. The excellent clinical efficacy, low host toxicity, along with the low cost of chloroquine accounts for its extensive clinical use.[7] In 1994, chloroquine was still the third most widely used drug in the world after aspirin and paracetamol.[8] Chloroquine is well tolerated in the treatment and prophylaxis of malaria. Only mild and moderate adverse effects have been identified, including gastrointestinal disturbance, headache, dizziness, blurred vision, insomnia, and pruritus.[9]



Chloroquine, 1

Figure 1.4 Structure of chloroquine.

The high selectivity of chloroquine action can be attributed to its target, the parasite-specific hemoglobin digestion process and its high rate of accumulation in malaria parasites. The unprotonated form of chloroquine can readily traverse the membranes of erythrocyte, parasite as well as digestive vacuole *via* simple diffusion. Once inside the acidic digestive vacuole whose pH is 4.8-5.2, chloroquine becomes protonated and is rendered impermeable to the membrane of digestive vacuole.[5] As a result, chloroquine is trapped in the acidic compartment of the parasite, where the concentration builds up.[5]

However, the value of chloroquine has been seriously eroded due to the development and spread of parasite resistance.[10] Currently, more than 80% of wild strains of *Plasmodium falciparum* are resistant to chloroquine.[4] Chloroquine resistance was

observed in Southeast Asia and South America at the end of the 1950s. In the late 1970s, the resistance also emerged in Africa. The accumulation of chloroquine in the acidic digestive vacuole significantly decreases in the case of chloroquine-resistant strains due to the *Plasmodium falciparum* chloroquine resistance transporter (PfCRT).[3] PfCRT, located in the digestive vacuole, belongs to the drug metabolite transporter superfamily. The mutation of the PfCRT gene in chloroquine-resistant strains results in the expression of the mutated PfCRT which is able to recognise and facilitate the efflux of chloroquine from the digestive vacuole.[5]

Owing to the global spread of chloroquine resistance, numerous attempts have been made to develop effective and safe agents for the treatment of chloroquine-resistant parasites. One of the most successful strategies to circumvent drug resistance is the modification of the aminoalkyl group in chloroquine, resulting in a series of chloroquine derivatives **2**-**5** (Figure 1.5). The variation of the side chain imparts activity against chloroquine-resistant *Plasmodium falciparum* to these analogues,[10] which is attributed to the fact that these derivatives cannot be recognised by the muted PfCRT, leading to the escape of these analogues from the structure-specific efflux.[5] AQ-13 (**2**, Figure 1.5) has been selected as an antimalarial candidate drug for clinical trials. The Phase I trial revealed a similar safety profile of AQ-13 compared with chloroquine. No serious toxic effects were identified in this study. Only moderate side effects occurred to some volunteers, including headache, dizziness and gastrointestinal symptoms such as nausea, vomiting, and diarrhea.[11] Commenced in 2013, the Phase II clinical trial of AQ-13 is still underway.

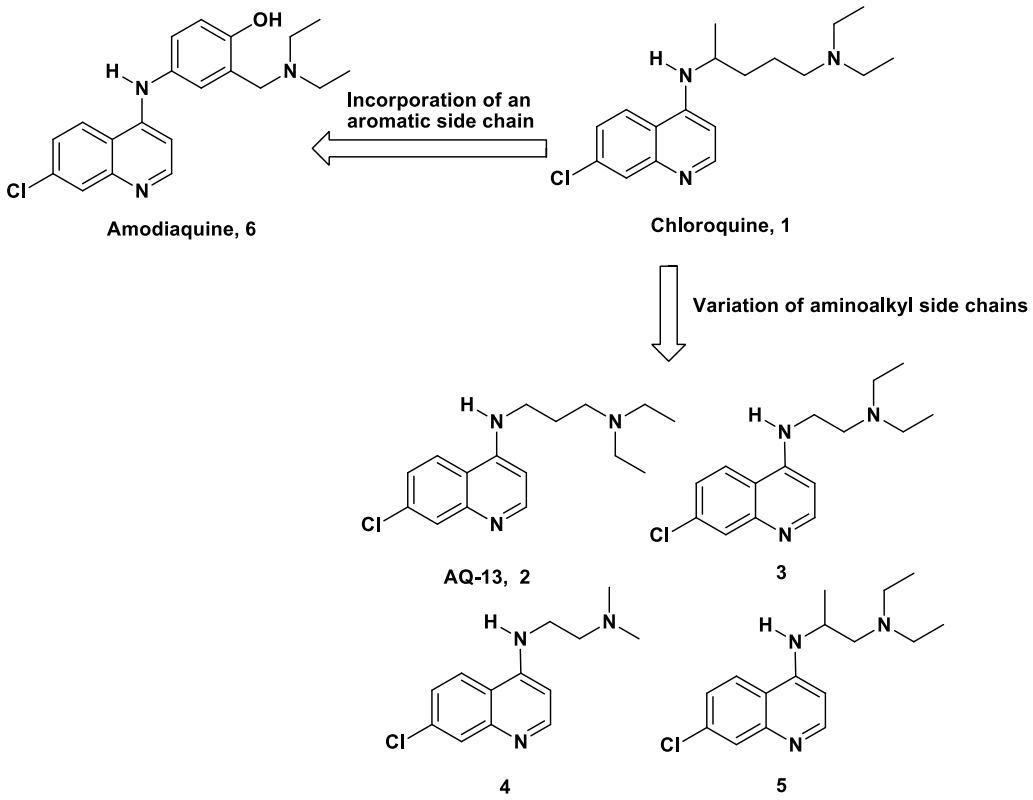


Figure 1.5 Chloroquine analogues with modified side chains.

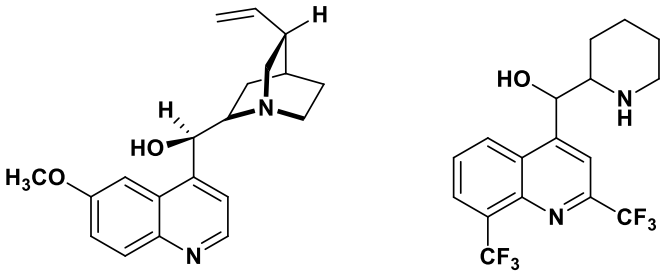
Compound	**1**	**2**	**3**	**4**	**5**
IC_{50} (NF54) (nM)	16	18	24	22	25
IC_{50} (K1) (nM)	315	59	49	50	61

Table 1.1 Activity of chloroquine and its analogues.

Incorporation of an aromatic ring into the chloroquine side chain affords amodiaquine **6** (Figure 1.5), which has been proved to be more effective than chloroquine in treating many chloroquine-resistant strains of *Plasmodium falciparum*.[12] Amodiaquine is on the *World Health Organisation's List of Essential Medicines 2013*, a list of the most important medications needed in the basic health system.[13]

1.1.2.2 Arylamino-alcohols

Quinine **7** (Figure 1.6), the active component of cinchona bark which was introduced into Europe from South America in the 17th century, has been utilised for the treatment of malaria for over 350 years.[14] Quinine remained the first-choice antimalarial drug until World War II, when other drugs such as chloroquine with greater tolerance replaced it. Currently, oral quinine is used for the treatment of uncomplicated malaria, which is a disease not causing severe organ dysfunction. In the case of severe malaria, quinine is administered by intramuscular injection or intravenous infusion.[15] However, quinine displays multiple adverse effects, including nausea, headache, tinnitus, hearing impairment, dysphoria, and blurred vision. More importantly, it induces a significant increase in insulin secretion, giving rise to severe hypoglycaemia.[16] Investigations have confirmed that quinine acts by inhibiting polymerisation of the haems released during haemoglobin degradation, resulting in the death of parasite.[6]



Quinine, 7 Mefloquine, 8

Figure 1.6 Antimalarial arylamino-alcohols: quinine and mefloquine.

Mefloquine **8** (Figure 1.6), as a structurally simplified quinine molecule, is a blood schizonticide active against the erythrocytic stages of *Plasmodium falciparum* and *Plasmodium vivax*. Ninety of the ninety-two chloroquine-resistant *plasmodium* strains show to be sensitive to mefloquine, indicating the potent antimalarial activity of mefloquine.[12] Mefloquine is utilised not only for the treatment of malaria but also for the prophylaxis.[17,18] However, the resistance to mefloquine has also emerged and continues to spread, especially in Southeast Asia.[19] In addition, the usage of mefloquine

is associated with significant neuropsychiatric adverse effects, such as dizziness, headache, insomnia and even epilepsy, which severely limits its utilisation.[16] As a derivate of quinine, mefloquine is also believed to act as an inhibitor of polymerisation of the haems.[6]

Halofantrine **9** and pyronaridine **10** (Figure 1.7) are also representative of arylamino-alcohol antimalarial agents. They are both effective against chloroquine-resistant *plasmodium* strains.[12] Nevertheless, the severe cardiac toxicity of halofantrine, including ventricular arrhythmias, significantly restricts the widespread use.[9] As a consequence, halofantrine has been withdrawn from the list of the first-line drugs recommended for the treatment of malaria by the World Health Organisation.[20]

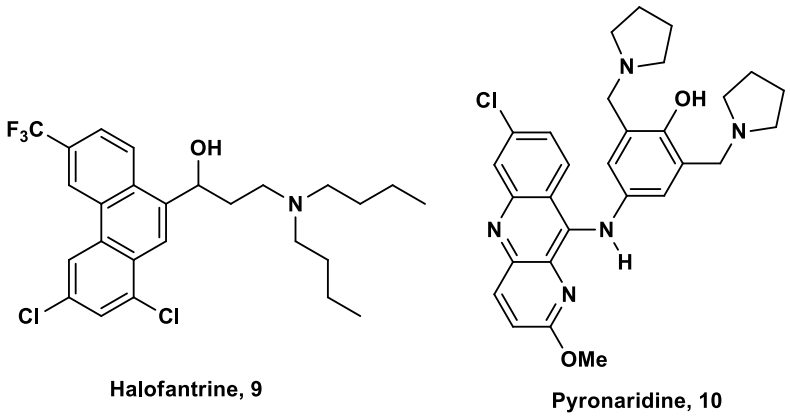


Figure 1.7 Antimalarial arylamino-alcohols: halofantrine and pyronaridine.

1.1.2.3 Artemisinin and its Derivatives

Artemisinin **11** (Figure 1.8) is an endoperoxide sesquiterpene lactone produced by aerial parts of *Artemisia annua*. The earliest report of *Artemisia annua* extracts being used in the treatment of malarial dates back to the 4th century AD, described in "The Handbook of Prescriptions for Emergencies" (*Zhouhou Beiji Fang*) by Ge Hong during the Jin Dynasty.[21] Artemisinin was isolated from the leaves of *Artemisia annua* in 1972.[22]

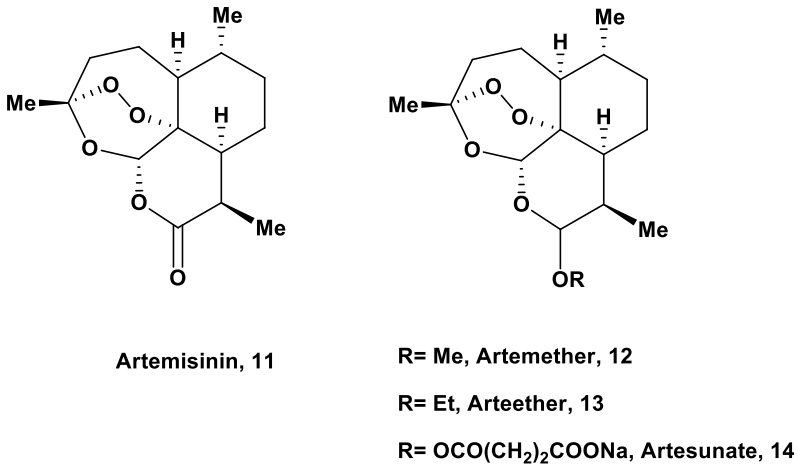


Figure 1.8 Artemisinin and its semisynthetic derivatives.

To date, artemisinin and its derivatives (**12-14**, Figure 1.8) have become one of the most efficacious classes of antimalarial agents and serve as the first-line treatment for multidrug-resistant malaria.[20] Artemisinin, a molecule poorly soluble in both water and oil, possesses a low bioavailability which limits its effectiveness. To address this problem, the three semisynthetic derivatives **12-14** were developed. Two of these derivatives are oil-soluble ethers: artemether **12** and arteether **13**; the other is a water-soluble hemisuccinate: artesunate **14**. Artemisinin derivatives clear parasitemia more rapidly than all the other currently available antimalarial agents, and they are the only antimalarials, apart from the cinchona alkaloids, used to treat severe *falciparum* malaria.[23] Two multi-centre, open-label and randomised trials for the evaluation of the efficacy of artesunate in the treatment of severe malaria were undertaken, which demonstrated that intravenous artesunate offered a significant reduction in mortality compared with intravenous quinine for patients suffering from severe malaria.[24,25] On the basis of these trials, WHO revised its *Guidelines for the Treatment of Malaria* in 2010, establishing parenteral artesunate in preference to quinine as the therapy of choice for severe malaria.[20]

The endoperoxide bridge has been demonstrated to play a crucial role in the activity of artemisinin and its derivatives. Research reveals 10-deoxoartemisinin **15 (**Figure 1.9)

retains the antiplasmodial activity, whereas 1-carba-10-deoxoartemisinin **16** **(**Figure 1.9), in the absence of the endoperoxide functionality, is devoid of antimalarial activity.[26]

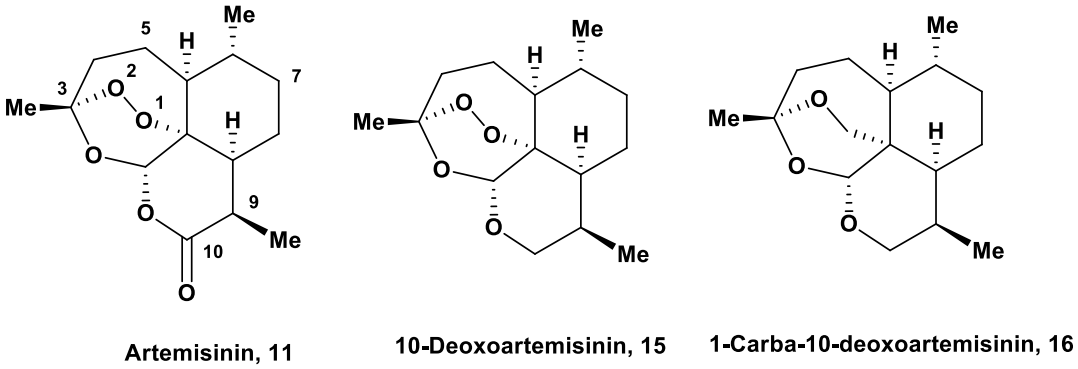


Figure 1.9 Artemisinin and deoxoartemisinins.

It has been demonstrated that artemisinins exert the antimalarial effect by the radical formation dependent on the endoperoxide moiety. In the presence of the haem generated in the haemoglobin metabolism by parasite, the endoperoxide bridge of artemisinin is broken, followed by the formation of drug-derived carbon-centred radical.[27] A mechanism proposal for this process has been put forward (Figure 1.10).[28] The reductive activation of artemisinin by Fe^{II}-haem (Fe(II)PPIX) leads to the cleavage of the endoperoxide bond and affords the oxygen-centred radical **17**, with the simultaneous oxidation of the iron(II) centre in the haem. This radical is promptly converted to the carbon-centred radical **18** *via* homolysis of the C_3-C_4 bond.[28]

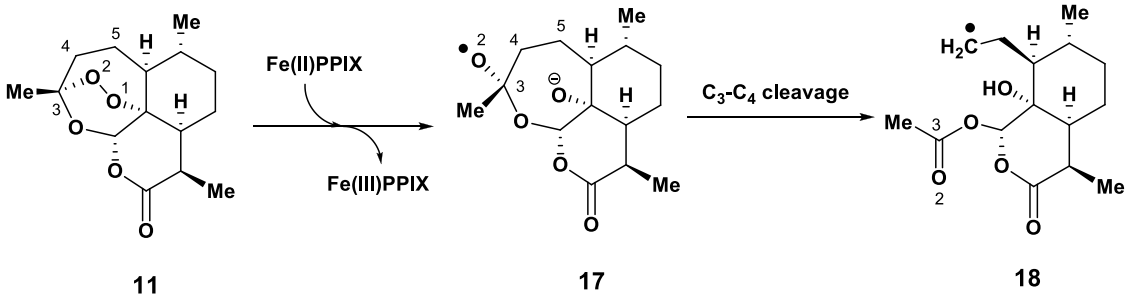


Figure 1.10 Mechanism proposal for the generation of carbon-centred radical.

This reactive alkyl radical is able to act as an alkylating agent toward haem to form an adduct responsible for the antimalarial activity (Figure 1.11).[27] Alkylation of haem by artemisinin *in vivo* has been attested by the successful identification of haem-artemisinin adduct in the spleen extracts of parasite-infected mice treated with artemisinin.[29] No haem-artemisinin adduct has been detected in the healthy mice treated with artemisinin. These observations demonstrate the alkylation of haem is a parasite-specific process in the action of artemisinin.[29] Haem-artemisinin adduct can inhibit the formation of haemozoin, which is the detoxification process of free haems by *plasmodium*, resulting in the death of parasite.[30]

Another proposal for the mode of action of artemisinin ascribes the antimalarial activity to its interference with the plasmodial haemoglobin catabolic pathway as well as the detoxification process of haem (Figure 1.11).[31] Aspartic and cysteine proteases are significant enzymes responsible for the proteolysis of haemoglobin in parasites. By the interaction with aspartic and cysteine proteases, artemisinin can disrupt the haemoglobin degradation, blocking the nutrient source for the parasite.[31] In addition, artemisinin is also able to interact with the haemozoin which is already formed in the parasite digestive vacuole, resulting in the breakdown of the haemozoin and the liberation of free haems.[31] Pandey has speculated this process might be related to the collapse of bond between iron and carboxylate in hemozoin caused by artemisinin.[31] The build-up of the toxic haems gives rise to the death of parasite.

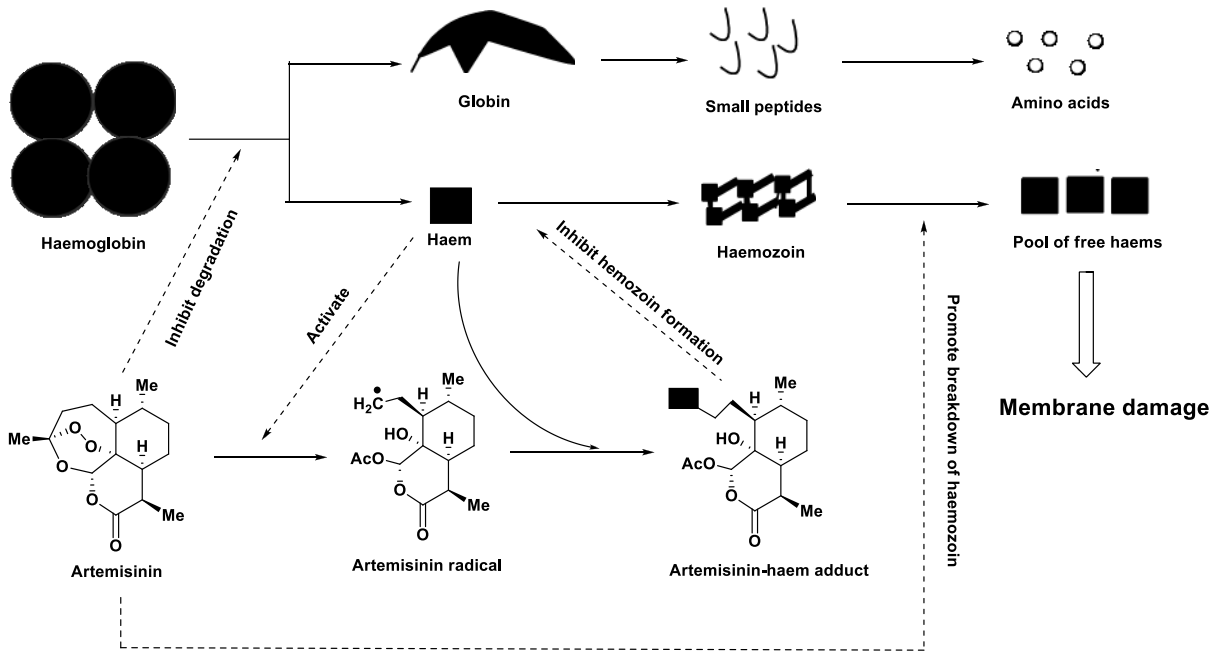
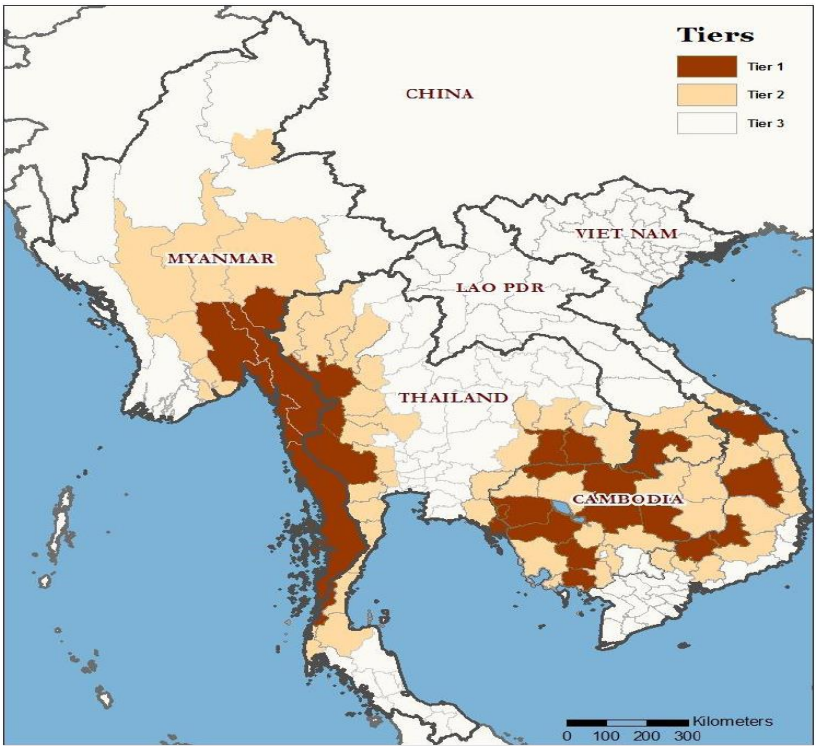


Figure 1.11 Comprehensive action mode of artemisinin.

Compared to other commonly employed antimalarial drugs, artemisinin derivatives have an excellent safety profile at the dosage recommended for treatment of a single malaria episode (4 mg/kg/day for 3 days).[32] Only mild and infrequent adverse effects including nausea, vomiting, anorexia and dizziness have been reported.[33] Nonetheless, higher doses administered over longer periods (6 mg/kg/day for 7 days) display a high rate of neutropenia (19%), suggesting the administration of artemisinins should strictly comply with the recommended dosage.[33]

The wide use of artemisinins have substantially reduced global morbidity and mortality from malaria.[4] Nevertheless, the prospect for the elimination of malaria is threatened by the emergence of artemisinin resistant *Plasmodium falciparum*.[23,34] The resistance has been confirmed across the five countries of the Greater Mekong subregion up to September 2014 (Figure 1.12).[34] Moreover, the relatively high cost and erratic supply of the natural artemisinin from plants is another key problem limiting the utilisation of artemisinins.[3]



TIER I: Areas with credible evidence of artemisinin resistance;

TIER II: Areas with significant inflows of migrant populations from tier I areas or shared borders with tier I areas;

TIER III: *Plasmodium falciparum* endemic areas without evidence of artemisinin resistance

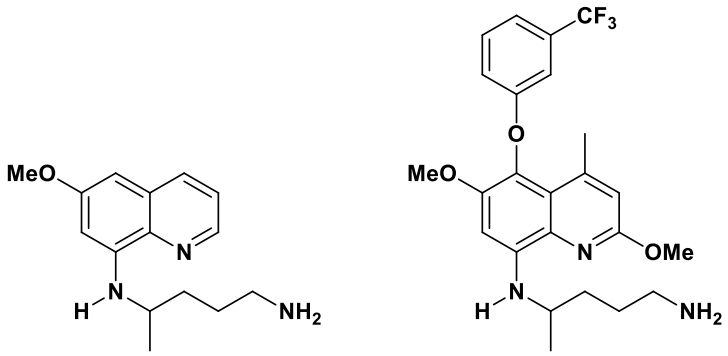
Figure 1.12 Tier map of the Greater Mekong subregion with artemisinin resistance.[34]

To halt and contain the spread of artemisinin resistance, World Health Organisation launched the *Global Plan for Artemisinin Resistance Containment* (GPARC) in 2011 to promote the application of artemisinin-based combination therapy. For instance, amodiaquine/artesunate fixed-dose combination has become a new effective medication for the treatment of *Plasmodium falciparum* malaria in Africa.[35] In addition, the combination therapy of artesunate and mefloquine has also been proved to be a safe, well tolerated and effective regimen in the treatment of multi-drug resistant *falciparum* malaria and is widely used in Southeast Asia.[36]

1.1.2.4 8-Aminoquinolines

Primaquine **19** (Figure 1.13) distinguishes itself from other antimalarial agents, as it displays activity against the hypnozoites and the sexual gametocytes of all the five *Plasmodia* infecting humans. Primaquine is also effective on the asexual erythrocytic stage of *Plasmodium vivax, Plasmodium ovale, Plasmodium malariae* and

Plasmodium knowlesi, whereas it is only weakly active against the erythrocytic stage of *Plasmodium falciparum*.[37] Owing to its unique feature, primaquine is widely used in prophylaxis and treatment of *Plasmodium vivax* and *Plasmodium ovale*.[37] By clearing the latent hypnozoites developed in the hepatic stage of these two *Plasmodia*, primaquine is able to prevent the relapse of *Plasmodium vivax* and *Plasmodium ovale*, which occurs months or even years after the initial infection.[38]



Primaquine, 19 **Tafenoquine, 20**

Figure 1.13 8-Aminoquinoline antimalarial agents: primaquine and tafenoquin.

In addition, the application of primaquine as a gametocytocide to reduce the transmission of *falciparum* malaria is also recommended by World Health Organisation.[39] Gametocytes of *Plasmodium falciparum* are relatively insensitive to artemisinins, so patients with transmissible gametocytes may remain infectious to mosquitoes for many days after receiving artemisinin-based combination therapy. Addition of primaquine to the artemisinin-based combination therapy can clear mature gametocytes effectively, blocking the infection of parasites to mosquitoes which serve as malaria vectors.[38] Notwithstanding these advantages, the major problem of primaquine limiting its use is the serious toxicity towards patients with glucose-6-phosphate dehydrogenase deficiency.[37] Therapeutic or higher doses of primaquine can induce acute haemolytic anemia or fatal haemolysis in G6PD-deficient individuals. In Arab populations living in malaria-risk areas, more than 30% of the patients show evidence of this deficiency in general, with an incidence of about 10-20%.[16]

Tafenoquine **20** (Figure 1.13), developed by Walter Reed Army Institute of Research in USA, is a primaquine analogue with a longer elimination half-life (14 days compared with 4 hours for primaquine). Tafenoquine is more potent against erythrocytic stage of *Plasmodium falciparum* than primaquine and also exhibits greater transmission-blocking activity.[37] However, tafenoquine possesses a similar haemolytic toxicity in G6PD-deficient patients. Phase-IIb studies are under way to determine risks and optimise dosing currently.[37]

1.1.2.5 Antifolates

Antifolates **21**-**27** (Figure 1.14) inhibit the synthesis of parasitic deoxythymidylate, and thereby halt the synthesis of parasitic DNA.[5] There are two classes of antifolates applied to the treatment of malaria: dihydrofolate reductase (DHFR) inhibitors such as pyrimethamine **21**, proguanil **22** and chlorproguanil **23** (Figure 1.14); dihydropteroate synthase (DHPS) inhibitors including sulfadoxine **24** and dapsone **25** (Figure 1.14). A dihydrofolate reductase inhibitor is usually used in combination with a dihydropteroate synthase inhibitor for the synergistic effect in clinical practice.

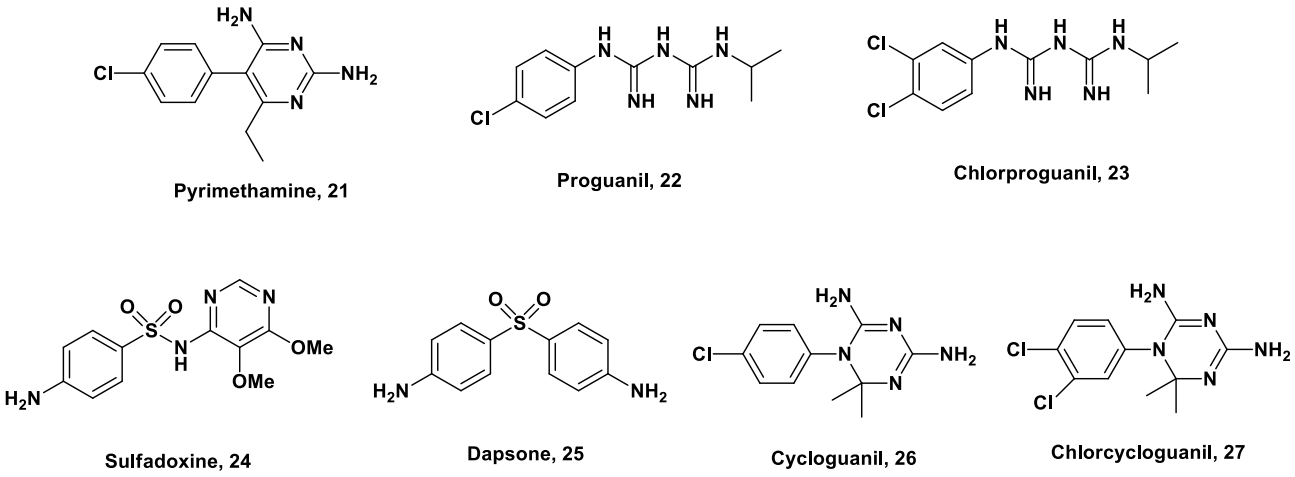


Figure 1.14 Antifolate antimalarial agents: DHFR and DHPS inhibitors.

Folate derivatives play a vital role in the biosynthesis of deoxythymidylate (dTMP). Mammals can obtain dihydrofolic acid from their diet, whereas malaria parasites completely depend on the biosynthesis of dihydrofolic acid from simple precursors to accomplish the *de novo* synthesis pathway of dTMP, which is critical to the parasites' survival.[40] As shown in Figure 1.15, 7,8-dihydro-6-hydroxymethylpterin pyrophosphate **28** (Figure 1.15) is condensed with *p*-aminobenzoic acid **29** to yield dihydropteroate **30** under the action of dihydropteroate synthase. Dihydrofolate **32** derived from the coupling between glutamic acid and dihydropteroate enters the folate cycle. In the presence of dihydrofolate reductase, dihydrofolate **32** is reduced to tetrahydrofolate **33**, which undergoes hydroxymethyl transferase-mediated conversion to yield 5,10-methylenetetrahydrofolate **36**. With 5,10-methylenetetra- hydrofolate serving as the methyl donor, the conversion of dUMP to dTMP is finally achieved under the action of thymidylate synthase. Dihydropteroate synthase is completely absent in humans, and the structure of protozoal dihydrofolate reductase is significantly different from that of the human enzyme, both of which set a stage for the development of selective inhibitors.[40]

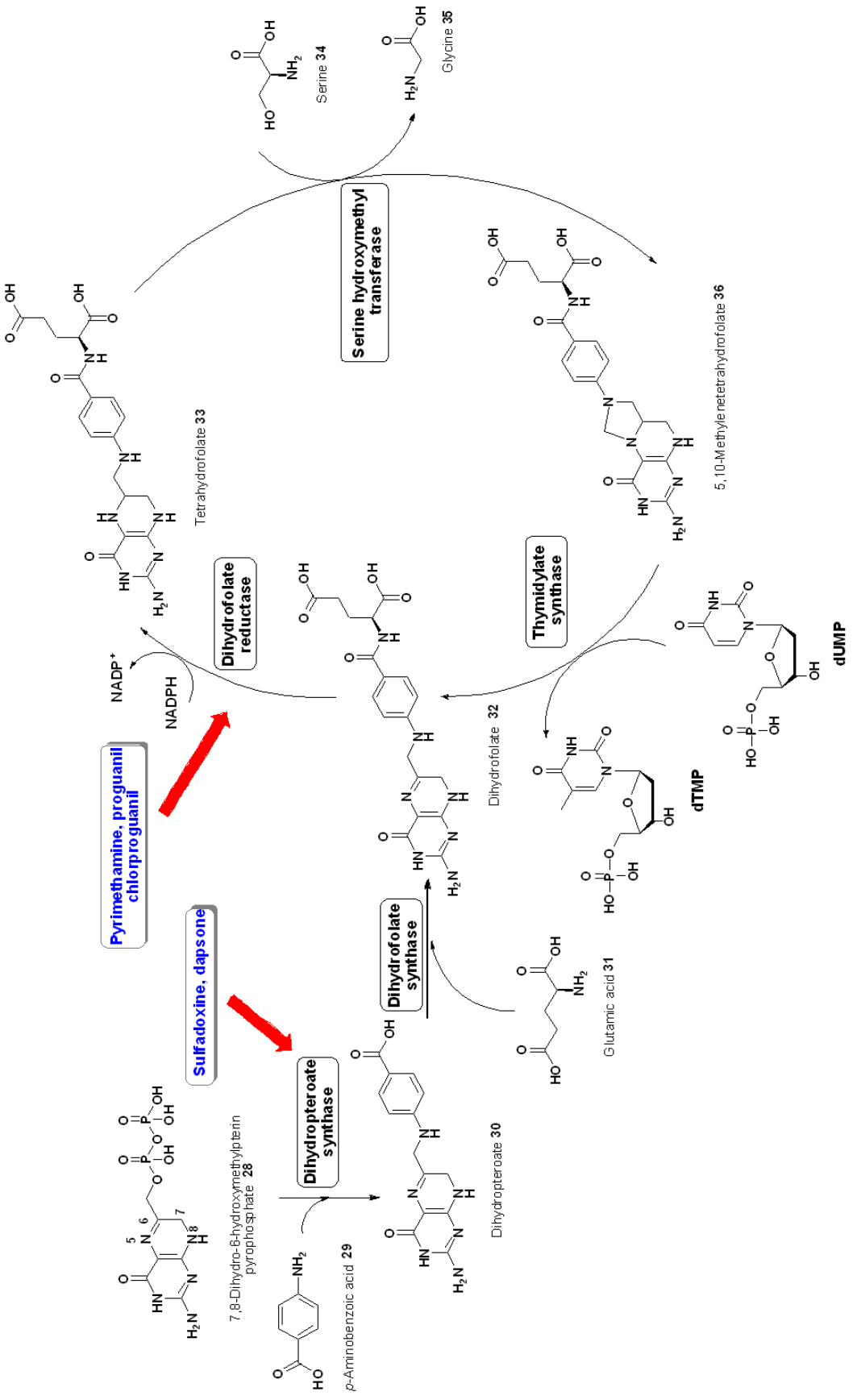


Figure 1.15 Folic acid metabolic pathway and its antagonists.

Owing to their structural resemblance to *p*-aminobenzoic acid, sulfadoxine **24** and dapsone **25** exert their activity by mimicking the normal substrate *p*-aminobenzoic acid to competitively bind to dihydropteroate synthase and block the biosynthesis of dihydropteroate **30**, resulting in the disruption to DNA synthesis (Figure 1.15).[5] It is the absence of dihydropteroate synthase in mammalian cells that renders sulfadoxine and dapsone selectively toxic towards malaria parasites.

Dihydrofolate reductase is another important enzyme targeted by inhibitors towards folic acid metabolic pathway. Possessing a high affinity to dihydrofolate reductase, pyrimethamine **21** is able to compete with dihydrofolate to bind tightly to the enzyme, impeding the conversion of dihydrofolate to tetrahydrofolate, thus blocking the synthesis of dTMP (Figure 1.15). Proguanil **22** and chlorproguanil **23** are prodrugs metabolised into the active components cycloguanil **26** and chlorcycloguanil **27** respectively, which also act by inhibiting reduction of dihydropteroate. The structural distinction between the dihydrofolate reductase in protozoans and its counterpart in mammals makes the DHFR inhibitors selectively recognise the protozoal enzyme and exert their activity against *Plasmodia* instead of humans.[5]

Pyrimethamine-sulfadoxine is a drug combination with two antifolates towards two different enzymes respectively. It has been extensively used for the treatment of *falciparum* malaria in Africa, including many least developed countries, due to the low cost of these drugs.[41] However, increased resistance to pyrimethamine-sulfadoxine has given rise to a decline in its effectiveness.[41] Moreover, the adverse effects of this combination is another issue requiring concerns. Higher doses of pyrimethamine-sulfadoxine cause megaloblastic anemia and toxic epidermal necrolysis.[16]

To tackle the problem of resistance towards pyrimethamine-sulfadoxine, LapDap® combining dapsone and chlorproguanil was developed by GlaxoSmithKline and

approved by the UK Medicines and Healthcare Products Regulatory Agency (MHRA) for the treatment of uncomplicated *Plasmodium falciparum* malaria in 2003.[42] The double-blind, randomised trial undertaken in Africa has manifested dapsone-chlorproguanil possesses greater efficacy than pyrimethamine-sulfadoxine.[41] Unfortunately, dapsone-chlorproguanil combination was withdrawn from the market in 2008 due to the incidents of drug-induced significant reductions of haemoglobin levels and haemolytic anaemia in patients with inherited glucose-6-phosphate dehydrogenase deficiency.[42]

A good number of antimalarial drugs have been discovered and developed in the 20th century, saving numerous lives all over the world. Nevertheless, the rapid spread of drug resistance has significantly eroded the value and reduced the effectiveness of these established antimalarial drugs. In addition, severe adverse effect caused by some of the antiplasmodial drugs is another issue limiting their wide application. Given the various problems encountered in the treatment of malaria, the development of novel effective, safe, and affordable drugs for the control and prevention of malaria is still one of the most pressing public health priorities worldwide.

1.1.3 Novel Antimalarial Azafluorenone Alkaloids

During a drug discovery programme aimed at the identification of new antimalarial leads from a natural product library, 5-hydroxy-6-methoxy-1-methyl-4-azafluoren-9-one **37** and 5,8-dihydroxy-6-methoxy-1-methyl-4-azafluoren-9-one **38**, isolated from the Australian tree *Mitrephora diversifolia*, were shown to possess antimalarial activity (Figure 1.16).[43]

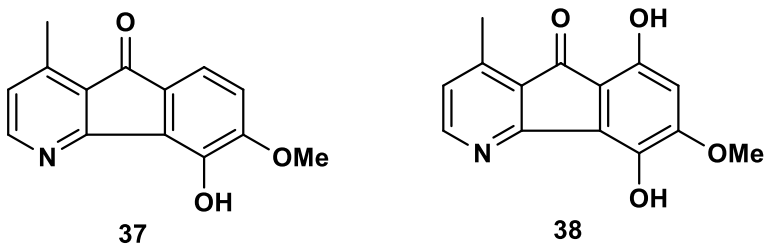


Figure 1.16 Novel antimalarial azafluorenone alkaloids.

When tested against two different strains of the *Plasmodium falciparum*, chloroquine-sensitive strain 3D7 and chloroquine-resistant strain Dd2, alkaloid **37** displayed IC_{50} values of 9.9 and 11.4 μM, respectively. In contrast, alkaloid **38** showed weak activity, with inhibition values of 87% and 80% at 120 μM against the 3D7 and Dd2 strains, respectively, and IC_{50} values for **38** were not provided.[43]

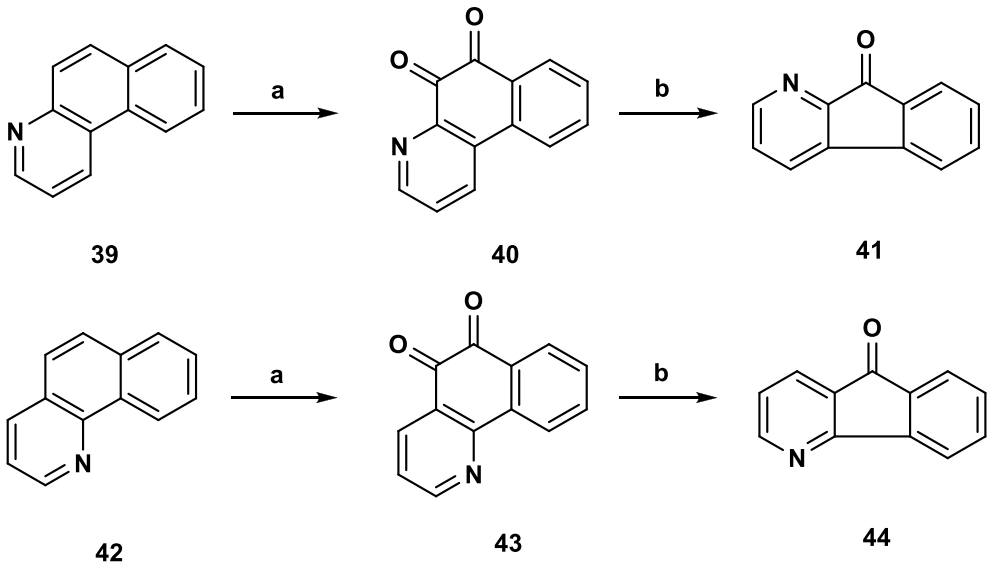
The core of these alkaloids is not present in the existing antimalarial drugs, providing an original entry point into the development of novel antiplasmodial agents. Possessing a number of positions available for further modifications, this structure affords a parent core for the design and development of a variety of derivatives with promising antimalarial activity.

1.1.4 Review of Synthetic Methods for Azafluorenones

Currently, the methods available for the synthesis of azafluorenones encompass three distinct approaches. The first method makes use of the degradation of nitrogen-containing fused compounds into tricyclic azafluorenones. The second approach is to increase the number of fused rings by annelation of indene or indane derivatives to construct the tricyclic system. The third strategy involves various intramolecular cyclocondensations of substituted arylpyridines or aroylpyridines.

1.1.4.1 Oxidative Degradation of Azaphenanthrenes

A good example utilising the first strategy is the two-step method developed by Kloc (Scheme 1.1).[44] Oxidation of 1-azaphenanthrene **39** and 4-azaphenanthrene **42** with iodine pentoxide yielded 1-azaphenanthrene-5,6-dione **40** and 4-azaphenanthrene-5,6-dione **43**. Treatment of the two diones with 10% sodium hydroxide solution at 85 °C afforded 1-azafluorenone **41** and 4-azafluorenone **44** in 88% and 70% yields, respectively. The limitation of this method is that azaphenanthrenes with complicated modifications are not commercially available.

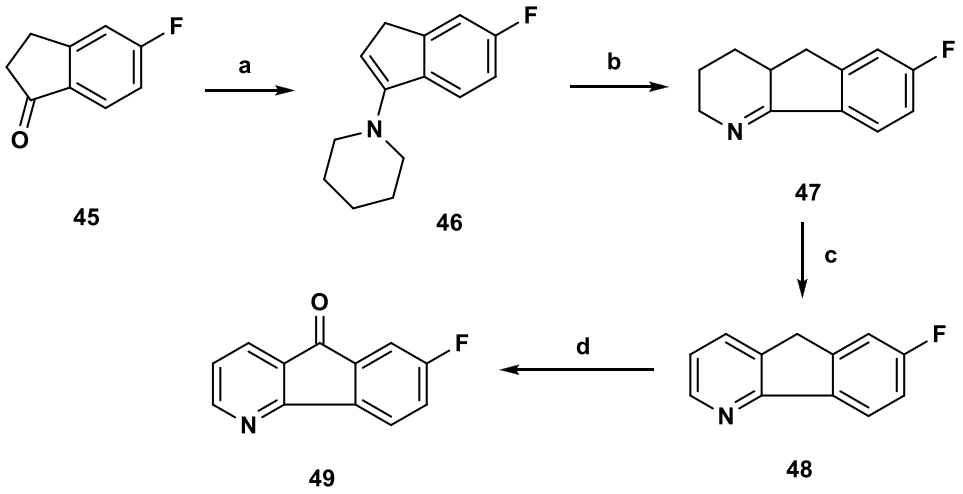


Scheme 1.1 *Reagents and conditions*: (a) I_2O_5, AcOH, reflux; (b) 10% NaOH aq, 85 °C.

1.1.4.2 Condensations involving Indene or Indane Derivatives

A variety of azafluorenone analogues have been synthesised from diverse indane and indene derivatives with suitable sources of nitrogen *via* a range of hetero-cyclocondensations.

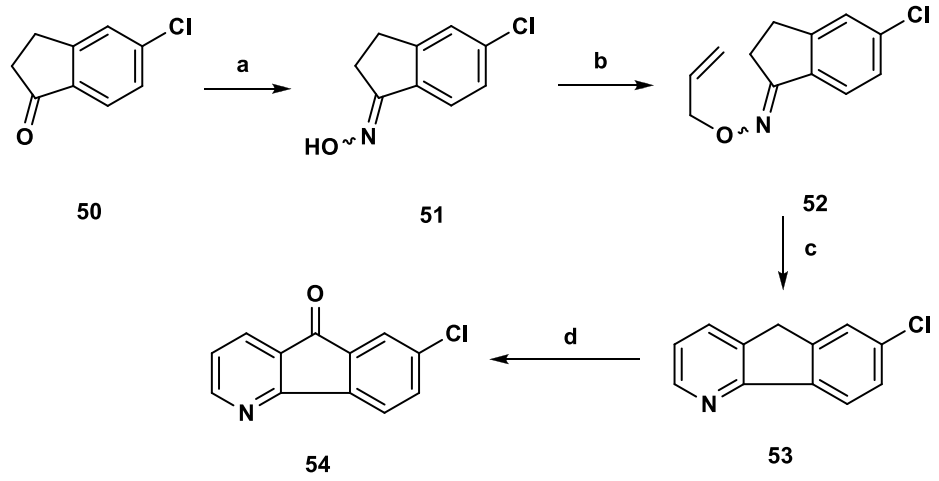
This strategy was firstly employed by Oliver in 1973 (Scheme 1.2).[45] Piperidine enamine **46** prepared from **45** is subjected to the one-pot two-step reaction involving a nucleophilic addition and a subsequent intramolecular nucleophilic substitution, which constructs the tricyclic framework. Further dehydrogenation of tetrahydroindenopyridine **47** provides **48** in a yield of 75%. The conversion of **48** to azafluorenone **49** was achieved in 36% yield *via* the oxidation by oxygen in the presence of benzyltrimethylammonium hydroxide. The major defect limiting the wide application of this method is the use of highly toxic nitrobenzene and harsh conditions (211 °C) for the Pd/C catalysed dehydrogenation.



Scheme 1.2 *Reagents and conditions*: (a) piperidine, TsOH, toluene, reflux; (b) $Br(CH_2)_3NH_2 \cdot HBr$, DMF, 100 °C; (c) 10% Pd/C, nitrobenzene, reflux; (d) O_2, benzyltrimethylammonium hydroxide, Py, rt.

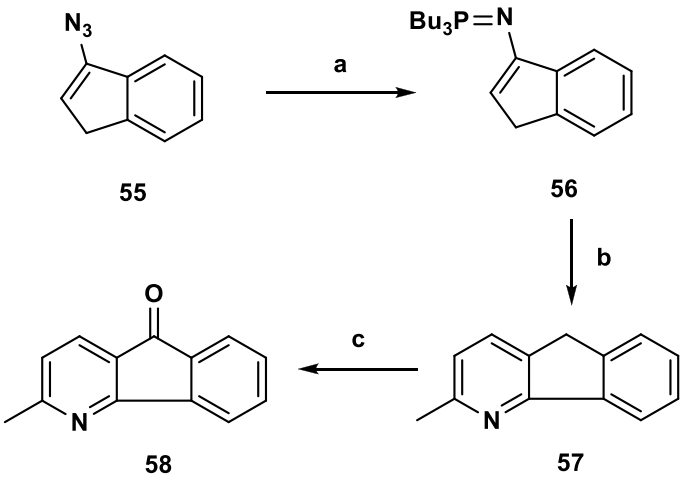
Another synthesis of halogenated azafluorenones from 5-halo-1-indanones is based on the thermolysis of *O*-allyl oxime ethers (Scheme 1.3).[46] 5-Chloro-1-indanone **50** was readily converted to the oxime ether **52** *via* base-catalysed *O*-alkylation of the corresponding oxime **51**. Thermolysis of **52** at 200 °C in a sealed tube provided azafluorene **53** in 15% yield, which was oxidised in the presence of oxygen and benzyltrimethylammonium hydroxide to furnish azafluorenone **54** in 33% yield. The major problem of this method lies on the low yield of the thermolysis (15%), which

greatly limits the utilisation of this approach.



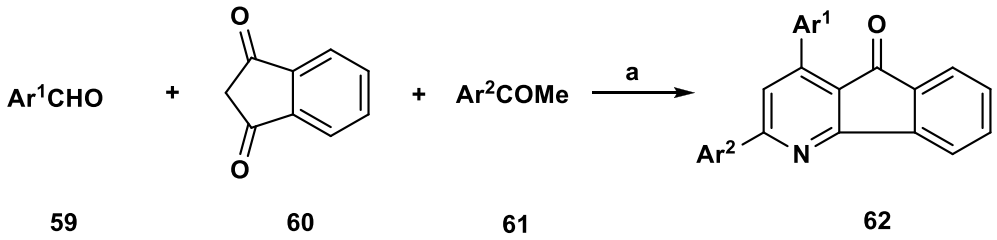
Scheme 1.3 *Reagents and conditions*: (a) $H_2NOH{\cdot}HCl$, EtOH, Py; (b) $CH_2{=}CHCH_2Br$, NaOEt, EtOH; (c) sealed tube, 200 °C; (d) O_2, benzyltrimethylammonium hydroxide, Py, rt.

An improved synthetic strategy with Michael addition and aza-Wittig reaction as the key steps was developed by Makoto (Scheme 1.4).[47] Treatment of 3-azidoindene **55** with tributylphosphine in toluene yielded tributyl(inden-3-ylimino)phosphorane **56**. The reaction of **56** with 3-butene-2-one involved a 1,4-addition and intramolecular aza-Wittig reaction, followed by the dehydrogenation to give azafluorene **57**. Treatment of **57** with chromium trioxide and *tert*-butyl hydroperoxide afforded azafluorenone **58** in a yield of 62%. In comparison to the previous methods initiating from indanone derivatives, this protocol avoids the extra high temperatures, broadening the substrate scope for this method.



Scheme 1.4 *Reagents and conditions*: (a) PBu_3, toluene, 0°C; (b) 3-butene-2-one, 10% Pd/C, toluene, reflux; (c) CrO_3, ^tBuOOH, CH_2Cl_2, rt.

A protocol for the preparation of azafluorenones employing the condensations of indane derivatives was developed by Tu (Scheme 1.5).[48]



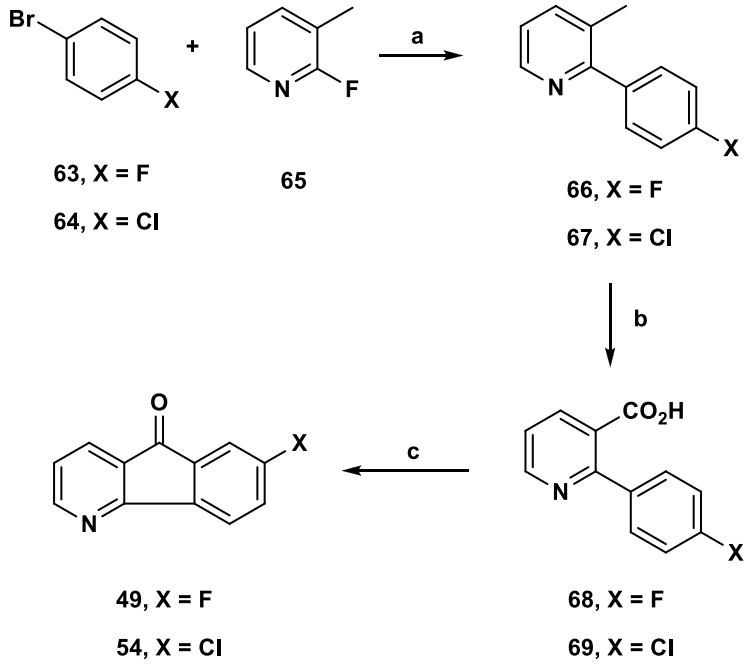
Scheme 1.5 *Reagents and conditions*: (a) NH_4OAc, DMF, 120 °C, microwave irradiation.

In the presence of ammonium acetate, the microwave-assisted one-pot condensation of aromatic aldehyde **59**, 1,3-indanedione **60** and aromatic ketone **61** *via* successive Michael reaction and nucleophilic additions afforded disubstituted azafluorenone **62** in 57-89% yields. This one-pot reaction affords a concise and efficient method for the synthesis of 1,3-diaryl-azafluorenones. However, only aromatic aldehydes and aromatic ketones have been utilised as the reactants for this method. More non-aromatic substrates need to be further examined to prove the versatility of this protocol.

1.1.4.3 Cyclocondensations of Substituted Arylpyridines or Aroylpyridines

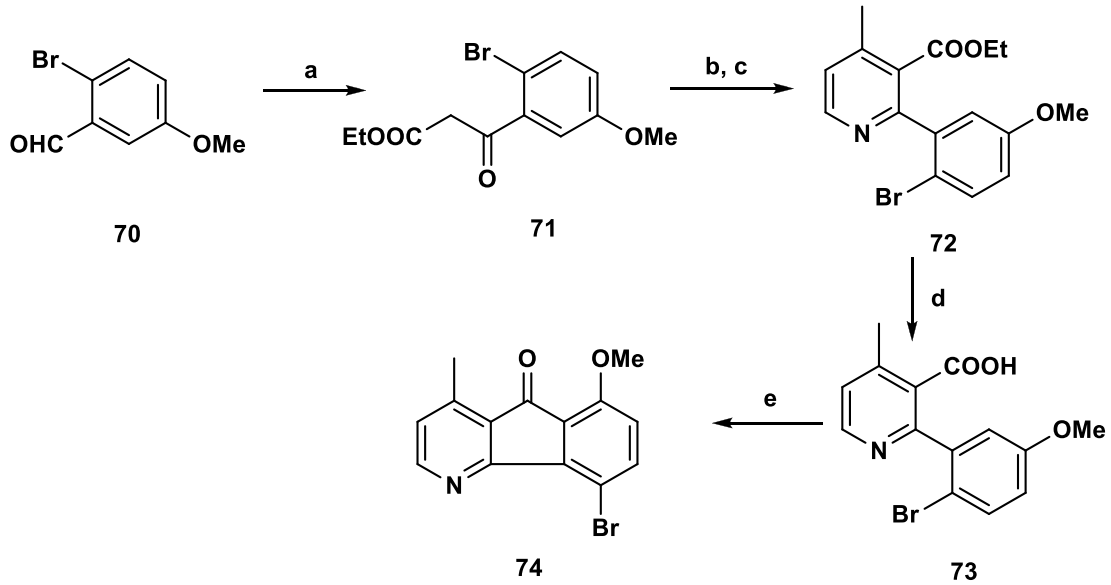
This strategy employs intramolecular cyclisations of the pyridines with aryl substitutions at the α-position or the pyridines with aroyl groups attached at the β-position The wide availability of diverse pyridine derivatives provides a broad scope for the synthesis of azafluorenones with various structural modifications.

In the synthesis of halogenated 4-azafluorenone developed by DuPriest, 2-aryl-3-methylpyridines **66** and **67** were accessed through the treatment of 2-fluoro-3-methylpyridine **65** with the corresponding aryllithium generated *in situ* (Scheme 1.6).[49] Subsequent oxidation of the azabiaryls **66** and **67** by potassium permanganate provided 3-nicotinic acids **68** and **69**. The intramolecular cyclisation of these two precursors was achieved in hot polyphosphoric acid, affording 4-azafluorenones **49** and **54** in 85% and 82% yields, respectively. This method follows a concise synthetic route employing the strategy of cyclocondensation of arylpyridine. Nonetheless, the utilisation of moisture sensitive lithium reagent brings some inconvenience to the experimental operation.



Scheme 1.6 *Reagents and conditions*: (a) *n*-BuLi, Et_2O, -40 ° C; (b) $KMnO_4$, water, reflux; (c) PPA, 200 °C.

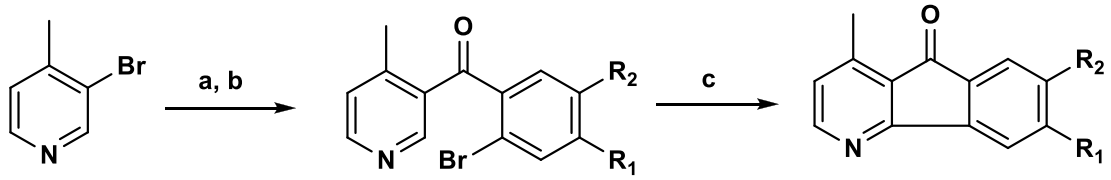
Bracher described another method based upon the cyclisation of arylpyridine (Scheme 1.7).[50] 2-Bromo-5-methoxybenzaldehyde **70** was converted to the β-keto ester **71** through the treatment with ethyl diazoacetate and tin(II) chloride. Michael addition of crotonic aldehyde to **71** followed by the reaction with hydroxylamine hydrochloride gave arylpyridine ester **72**. Saponification of this ester furnished the cyclisation precursor **73**. Nicotinic acid **73** was cyclised into azafluorenones **74** in a yield of 79%, under the catalysis of polyphosphoric acid at 130 °C. Compared with DuPriest's protocol,[49] the temperature for the PPA-catalysed cyclisation in this method is reduced to a lower temperature (130 °C), which has been demonstrated to be equally efficient for the intramolecular acylation. This refinement improves the functional group tolerance of this method and broadens the substrate scope applicable to this cyclisation, providing a viable approach for the synthesis of the antimalarial natural product **37** and its analogues. However, the use of the toxic reagent tin(II) chloride is the major defect of this protocol. It is necessary to make some improvement for the preparation of the arylpyridine before its application to the synthesis of alkaloid **37**.



Scheme 1.7 *Reagents and conditions*: (a) $N_2CHCOOEt$, $SnCl_2$, CH_2Cl_2, rt; (b) crotonic aldehyde, benzyltrimethylammonium hydroxide, dioxane, rt; (c) $H_2NOH{\cdot}HCl$, EtOH, reflux; (d) KOH,

EtOH, H_2O, reflux; (e) PPA, 130 °C.

Kraus developed an alternative approach utilising an intramolecular Heck reaction as the key step in the synthesis of azafluorenones (Scheme 1.8).[51] Different from the previous methods based on the α-arylpyridine, this protocol employs the carbonyl group to link the phenyl ring with the pyridyl moiety at the β-position prior to the connection at the α-position. The reaction of a substituted bromobenzaldehyde with the lithiopyridine derived from metal-halogen exchange afforded an alcohol intermediate, which was readily oxidised by manganese dioxide. The intramolecular Heck reactions of bromobenzophenones **76**-**78** provided azafluorenones **79**-**81** in 53%, 47% and 50% yields, respectively.



75

76, R_1 = R_2 = H

77, R_1 = R_2 = OMe

78, R_1 = H, R_2 = OMe

79, R_1 = R_2 = H

80, R_1 = R_2 = OMe

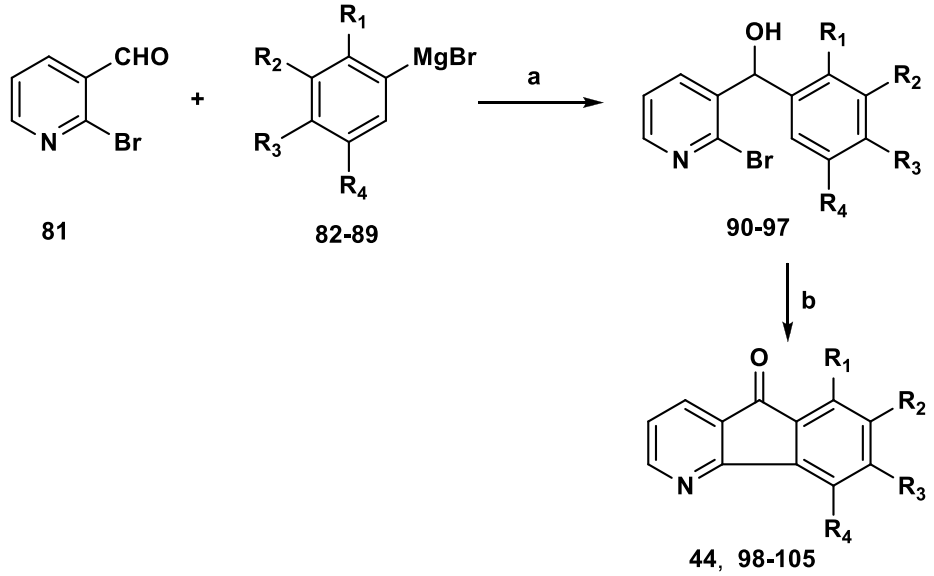
81, R_1 = H, R_2 = OMe

Scheme 1.8 *Reagents and conditions*: (a) *n*-BuLi, substituted bromobenzaldehydes, THF, -100 to -78 °C; (b) MnO_2, benzene, reflux; (c) $Pd(OAc)_2$, Na_2CO_3, DMF, 130 °C.

This protocol provides a concise route for the preparation of azafluorenones. Nevertheless, the strong basic condition required for the first step limits its application to the pyridine substrates bearing vulnerable functionalities such as carbonyl group, ester group and so forth. The low temperature (-100 °C) and the utilisation of highly moisture sensitive *n*-butyllithium also renders the experimental operation inconvenient.

Ray developed a protocol for the synthesis of azafluorenones *via* a Grignard reaction followed by an intramolecular Heck reaction (Scheme 1.9).[52] Treatment of 2-bromonicotinaldehyde **81** with Grignard reagents **82**-**89** provided the condensation

products **90-97** in 73-95% yields, which were subjected to the subsequent Heck reactions. In the presence of palladium acetate and sodium acetate, the intramolecular cyclisation was fulfilled at 100 °C to afford azafluorenones **44** and **98-105** in moderate to excellent yields (Table 1.2).



Scheme 1.9 *Reagents and conditions*: (a) ether, 0 °C; (b) $Pd(OAc)_2$, NaOAc, DMF, 100 °C.

Entry	R_1	R_2	R_3	R_4	Product	Yield (%)
1	H	H	H	H	**44**	95
2	OMe	H	H	H	**98**	78
3	H	H	Cl	H	**99**	75
4	H	Me	H	Me	**100**	93
5	H	Cl	H	Cl	**101**	74
6	H	Cl	Cl	H	**102**	68
7	Benzo		H	H	**103**	53
8	H	H	F	H	**104**	61

Table 1.2 Synthesis of azafluorenones *via* intramolecular Heck reactions.

Compared with the method developed by Kraus,[51] this protocol utilises Grignard reaction to replace the original condensation employing the lithiopyridine at the extra

low temperature (-100 °C), avoiding the inconvenience for the experimental operation.

1.1.5 Aim of the Project

Since only a preliminary study was conducted looking into the antimalarial activity of alkaloids **37** and **38** by Quinn,[43] our initial focus will be to accomplish the total synthesis of natural product **37**, with which more elaborate and deeper investigations into its antimalarial activity can be conducted. On the basis of the current methods available for the preparation of azafluorenones, a synthetic route will be developed towards alkaloid **37**. Possessing the structure with a number of positions available for modification, the parent core provides a good starting point for the development of a variety of derivatives. Having developed the synthetic route to the natural product, diverse analogues of alkaloid **37** will be prepared in a similar manner to explore the structure-activity relationship of the class of azafluorenones Moreover, it is also planned to explore the mechanism of action, which will set the stage for the further design and development of promising antimalarial agents.

1.2 Synthesis of Antimalarial Natural Product 37 and Azafluorenone Analogues

1.2.1 Design of Azafluorenone Analogues

As a classical bioisostere of hydroxyl group,[53,54] fluorine has been incorporated into

drug molecules to replace a hydroxyl group to ameliorate biological activity.[55] The unique natures of fluorine, including the high electronegativity, the ability of forming hydrogen bonds, the high lipophilicity of fluoroaryl groups and the great strength of carbon-fluorine bond, impart distinctive chemical and biological properties to organofluorine compounds. The introduction of fluorine into organic molecules usually improves the molecules membrane permeability and metabolic stability, and increases the affinity towards the protein targets.[56] Accordingly, a large number of organofluorine compounds exhibit enhanced biological activity compared to their non-fluorinated analogues.[56] Consequently, azafluorenone analogue **106**, with the 5-hydroxyl group replaced by a bioisosteric fluorine, was designed to mimic the natural product **37**, hoping to acquire enhanced antimalarial activity (Figure 1.17).

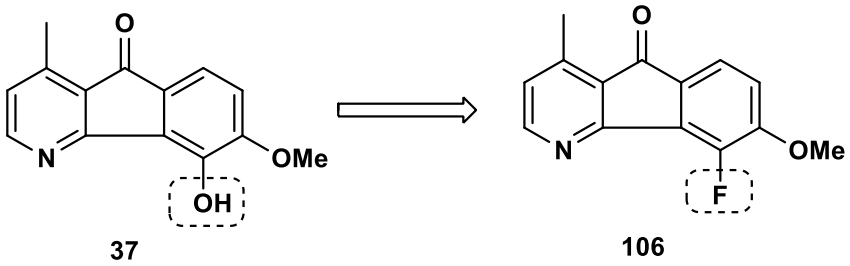


Figure 1.17 Structure of the fluorinated mimic of alkaloid **37**.

Alkaloid **38** has been proved to display a lower activity against *Plasmodium falciparum* (3D7 and Dd2 strains) than **37** (Figure 1.18),[43] implying that the 8-hydroxyl group in alkaloid **38** is not essential for the antimalarial activity. The presence of the 8-hydroxyl group might obstruct the interaction between the alkaloid and the target site. Thus, there is a question as to whether all of the functional groups present in alkaloid **37** are required for the antimalarial activity. Consequently, the simplified analogue **107** (Figure 1.19) would be synthesised to determine whether the 1-methyl-4-azafluoren-9-one core possesses antimalarial activity.

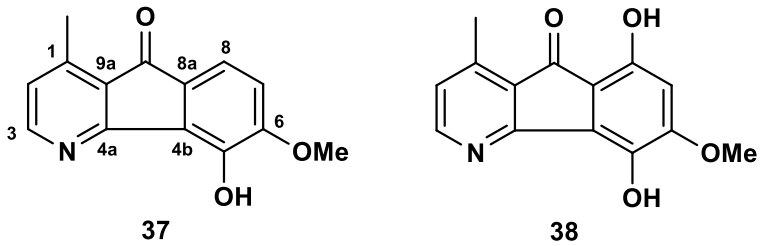


Figure 1.18 Structures of the antimalarial alkaloids **37** and **38**.

To investigate whether the 5-hydroxyl group or 6-methoxyl group is crucial for the antimalarial activity, analogues with 5-hydroxyl group and 6-methoxyl group deleted respectively were designed (azafluorenones **108** and **109**, Figure 1.19). With the purpose of probing into the interaction between the functional groups at the 5-position and the target site, it was decided to append electron-withdrawing and electron-donating groups to this position respectively, with azafluorenones **110** and **111** designed. Likewise, azafluorenones **112** and **113** were also designed to explore the interaction between the functional groups at the 7-position and the target site. By making these simplifications and modifications, we will identify the minimal active fragment possessing antimalarial activity and acquire a preliminary structure-activity relationship regarding the phenyl moiety.

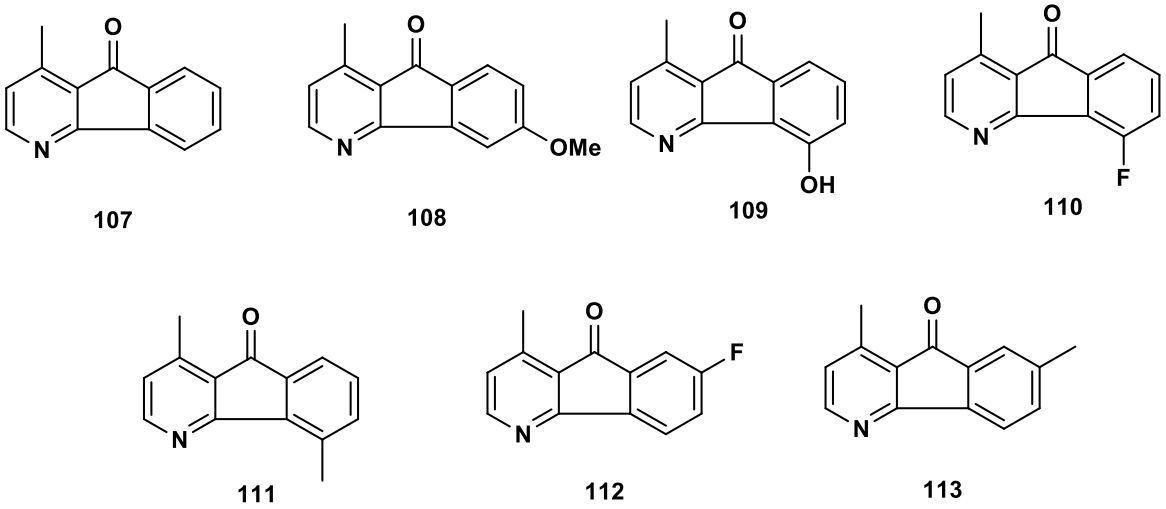


Figure 1.19 Structures of azafluorenones with modifications on the phenyl moiety.

To explore the structure-activity relationship around the pyridyl moiety, a series of analogues decorated on the pyridyl ring were designed based on commercially available nicotinates (Figure 1.20). To explore the role of the methyl group in the antiplasmodial activity, azafluorenone **114** with the methyl group moved to the 3-position was designed. To investigate the interaction between the functional groups at the 3-position and the target site, it was decided to append electron-withdrawing and electron-donating groups to this position respectively, with azafluorenones **115** and **116** designed. Likewise, azafluorenones **117** and **118** were also designed to probe the interaction between the functional groups at the 1-position and the target site.

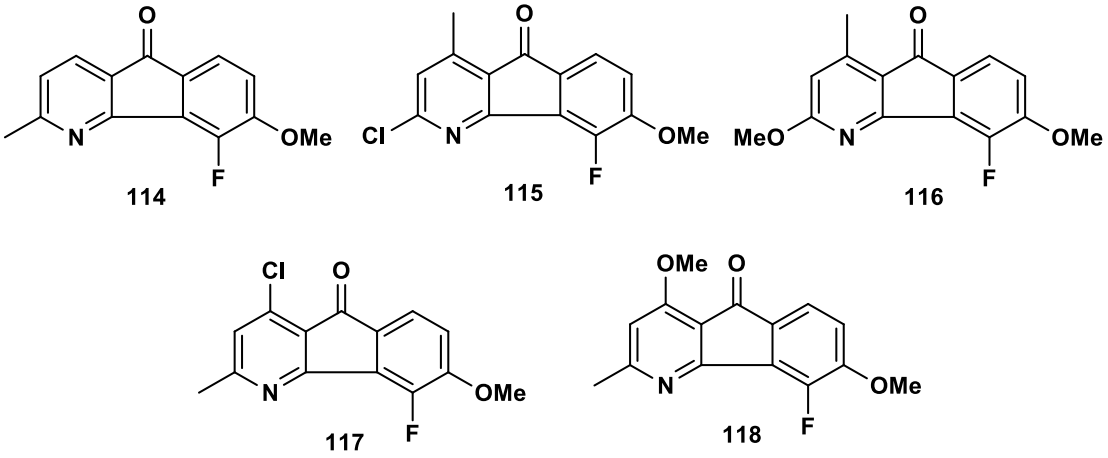


Figure 1.20 Structures of azafluorenones with modifications on the pyridyl moiety.

The structures of many currently and previously utilised antimalarial agents contain a common amino side chain (Figure 1.21). This amino side chain has been shown to play a vital role in the antiplasmodial activity of these compounds, facilitating the accumulation of the drugs in the digestive vacuoles of *Plasmodia*.[5]

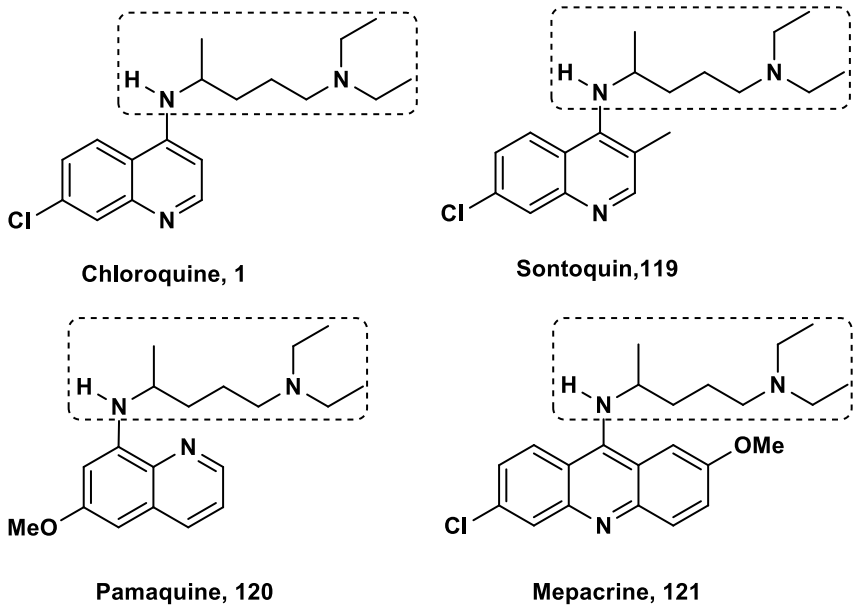


Figure 1.21 Structure of antimalarial drugs bearing a dialkyl tertiary amino group.

Modifications of the side chain of chloroquine, such as altering the length of the side chain, has been shown to impart activity against chloroquine-resistant strains for analogues **2-5** (Figure 1.22).[10] This is attributed to fact that analogues with the decorated side chains cannot be recognised by the muted *Plasmodium falciparum* chloroquine resistance transporter (PfCRT). The mutation of the gene coding PfCRT in chloroquine-resistant strains results in the expression of the mutated PfCRT which is able to recognise and facilitate the efflux of chloroquine from the digestive vacuole.[5] The variation of the side chain makes these derivatives not recognised by the mutated PfCRT, escaping from this structure-specific efflux.[5] Amodiaquine **6** (Figure 1.22), as a derivative of chloroquine, is an extensively used 4-aminoquinoline antimalarial drug. The replacement of the original amino side chain in chloroquine by the aromatic ring not only provides a higher activity against chloroquine-sensitive strains, but also renders it effective at inhibiting chloroquine-resistant strains at the erythrocytic stage.[3]

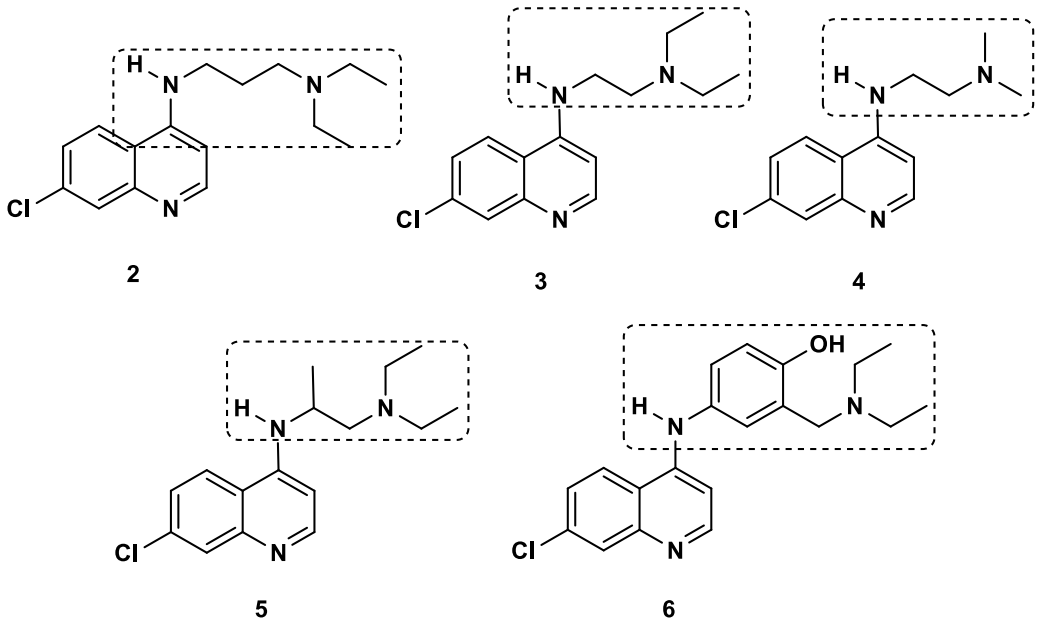


Figure 1.22 Structures of antimalarial molecules with different side chains.

Accordingly, it would be of value to conjugate these structural fragments with the azafluorenones to create hybrid molecules **122**-**126** (Figure 1.23) and investigate the effects of different side chains on the antiplasmodial activity of the azafluorenones. The introduction of a range of amino side chains would be anticipated to facilitate the accumulation of these modified azafluorenones in the parasite acidic digestive vacuole due to the increased basicity. The higher concentration of hybrid molecules present in the parasite might lead to enhanced antimalarial activity.

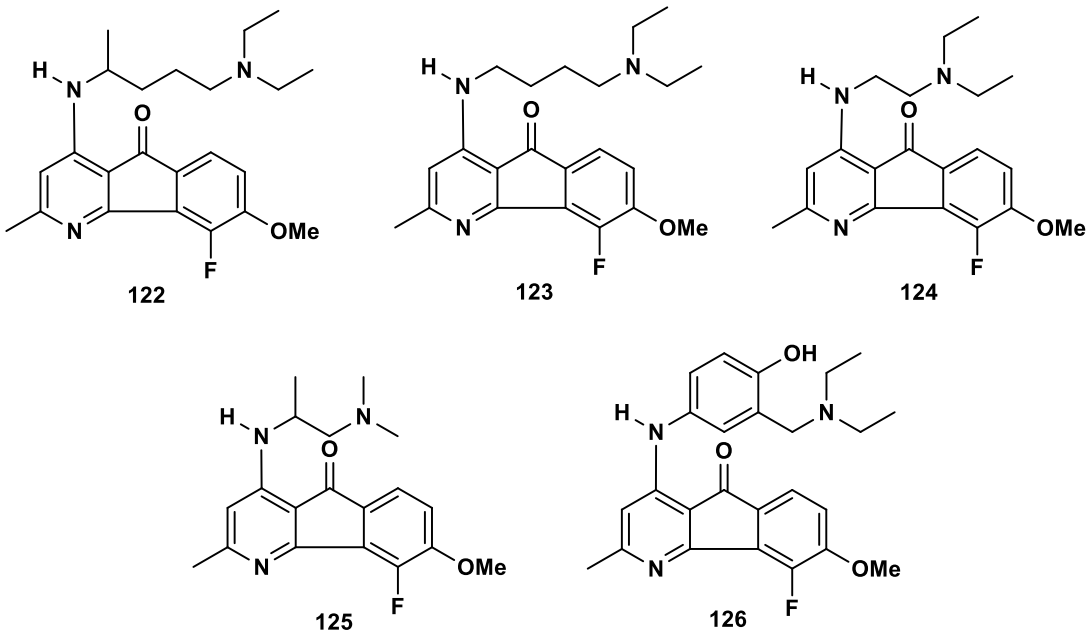
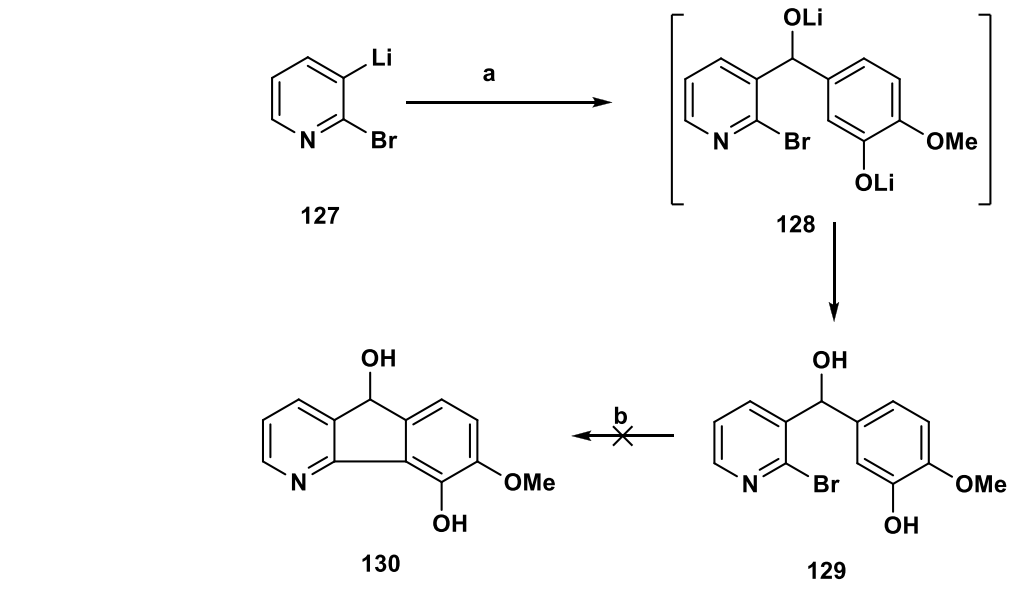


Figure 1.23 Structures of designed hybrid molecules.

1.2.2 Synthetic Routes towards Natural Prodcut 37

The attempt to synthesise a related derivative of antimalarial alkaloid **37** was described by Kraus,[51] employing an intramolecular Heck reaction as the key step. The condensation between 3-lithio-2-bromopyridine **127** and 3-hydroxy-4-methoxybenzaldehyde was successful. Nonetheless, subsequent cyclisation *via* the Heck reaction failed to afford the target tricyclic product **130** (Scheme 1.10).



Scheme 1.10 *Reagents and conditions*: (a) 3-hydroxy-4-methoxybenzaldehyde, THF, rt; (b) $Pd(OAc)_2$, Na_2CO_3, DMF, 130 °C.

Different from Kraus' strategy, our retrosynthetic analysis of natural product **37** is shown in Figure 1.24. The formal synthesis involves two key steps, the construction of the fused tricyclic ring system and the formation of azabiaryl **132**.

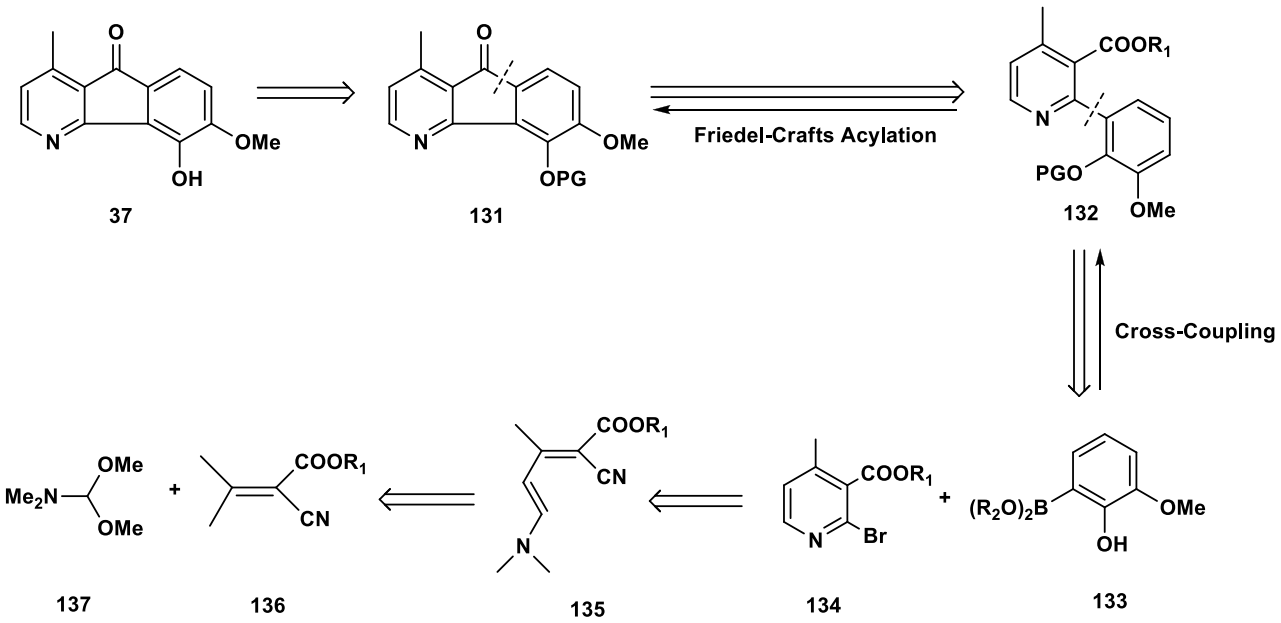
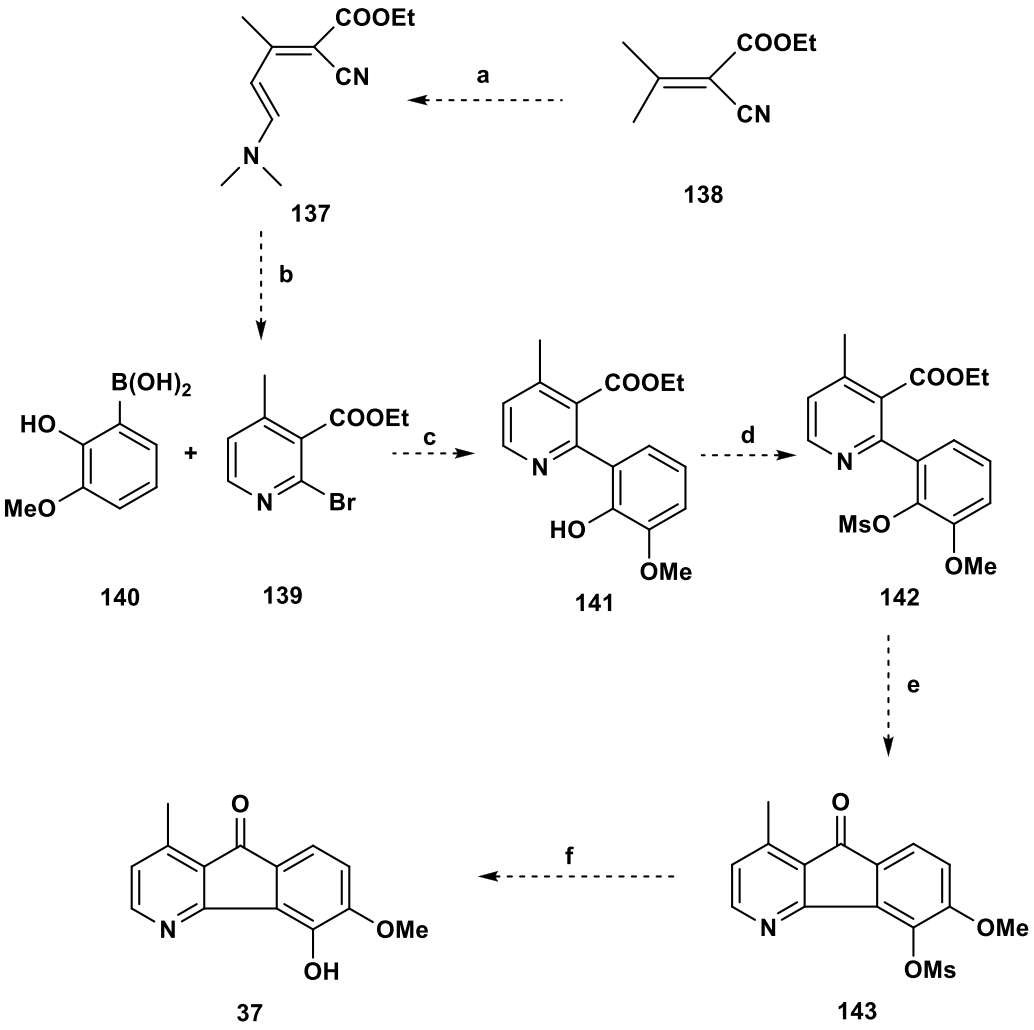


Figure 1.24 Retrosynthetic analysis towards alkaloid **37**.

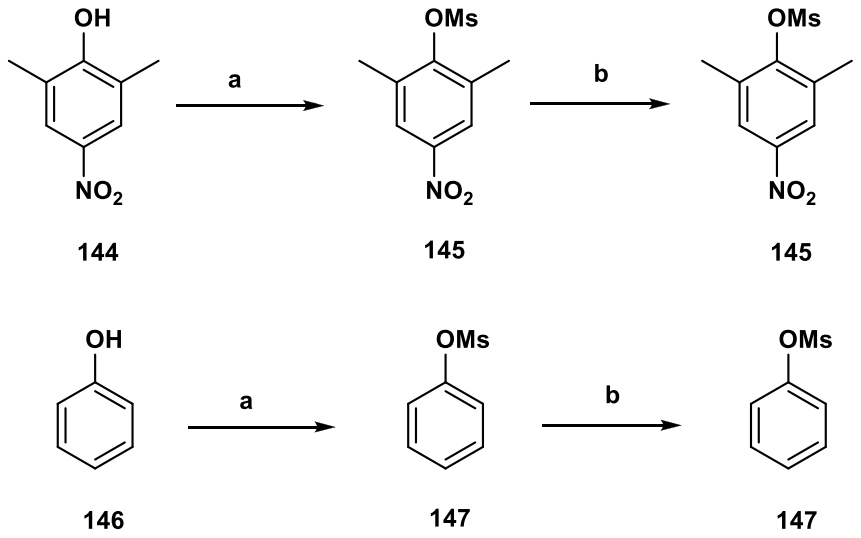
As an extensively utilised catalyst for intramolecular Friedel-Crafts acylation reactions,[57] polyphosphoric acid (PPA) would be applied to the intramolecular cyclisation, constructing the tricyclic ring parent core. The palladium-catalysed Suzuki-Miyaura cross-coupling reaction would be exploited to achieve the formation of the azabiaryl.[58] Since the polar and amphiphilic characters of arylboronic acids often render their isolation and purification difficult[59] and free hydroxyl groups do not obstruct the Suzuki cross-coupling,[58,60] it was planned to protect the free hydroxyl group after the cross-coupling reaction. The preparation of the decorated nicotinate would be fulfilled in two steps according to literature methods.[61]

The selection of the protecting group for the free hydroxyl group in precursor **132** is crucial. Since the PPA-catalysed cyclisation occurs at 140 °C, a protecting group that is completely stable under the acidic conditions and at high temperature is needed. Sulfonates are stable under acidic conditions and widely used for the protection of phenols.[62] Accordingly, it was decided to employ the methanesulfonyl (Ms) protecting group in the synthesis. After the intramolecular Friedel-Crafts acylation, the Ms group would be removed using sodium hydroxide in methanol/water solution.[63] The complete synthetic route towards alkaloid **37** is shown in Scheme 1.11. This synthetic route is sufficiently versatile to achieve the preparation of alkaloid analogues starting from appropriate nicotinates and arylboronic acids.



Scheme 1.11 *Reagents and conditions*: (a) DMF acetal, EtOH, reflux; (b) 30% HBr-AcOH, AcOH, 55 °C; (c) $Pd(PPh_3)_4$, THF, 2 M Na_2CO_3 aq, 80 °C; (d) MsCl, Et_3N, CH_2Cl_2, rt; (e) PPA, 140 °C; (f) NaOH aq, MeOH, 60 °C.

To confirm the stability of aryl methanesulfonate esters under the proposed PPA-catalysed cyclisation condition, two model reactions were carried out. The mesylations of 2,6-dimethyl-4-nitrophenol **144** and phenol **146** were achieved in the presence of methanesulfonyl chloride and triethylamine, affording **145** and **147** in 81% and 90% yields, respectively (Scheme 1.12). The mesylated products **145** and **147** were then stirred in polyphosphoric acid at 200 °C, a higher temperature than required for the PPA-catalysed cyclisation. There was no decomposition detected for either of the two methanesulfonate esters after 6 hours, indicating that aryl methanesulfonate esters can tolerate these reaction conditions.

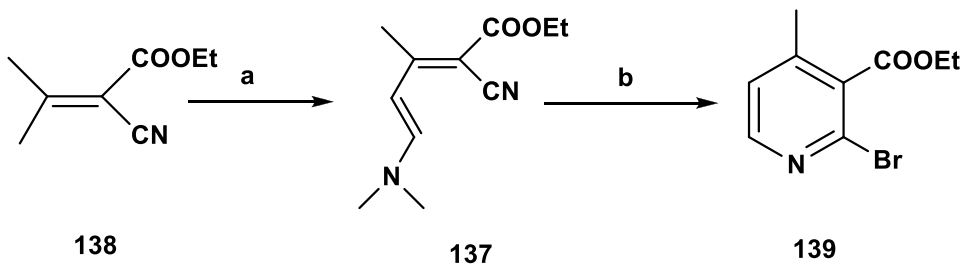


Scheme 1.12 *Reagents and conditions*: (a) MsCl, Et_3N, CH_2Cl_2, rt, 6 h; (b) PPA, 200 °C, 6 h.

1.2.3 Synthesis of Parent Core 1-Methyl-4-azafluoren-9-one

Prior to the synthesis of natural product **37**, it was decided to prepare the simplified azafluorenone **107** to confirm the feasibility of the proposed synthetic route.

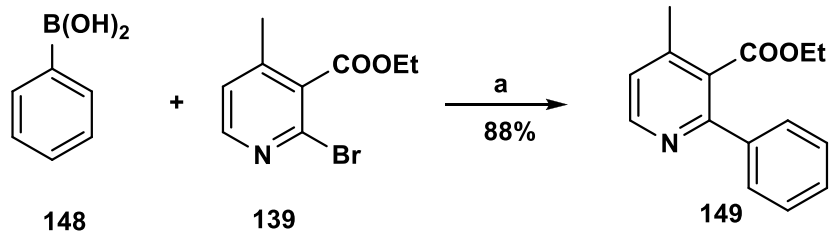
The reaction of ethyl 2-cyano-3-methyl-2-butenoate **138** with *N*,*N*-dimethylformamide dimethyl acetal afforded diene **137**. The electrophilic addition of hydrogen bromide to the nitrile group followed by an acid-catalysed cyclisation provided the decorated nicotinate **139** in a good yield of 62% over two steps (Scheme 1.13).[61]



Scheme 1.13 *Reagents and conditions*: (a) DMF acetal, EtOH, reflux; (b) 30% HBr-AcOH, AcOH, 55 °C.

As one of the most efficient methods for the construction of carbon-carbon bonds, the Suzuki-Miyaura cross-coupling reaction plays a vital role in synthetic organic chemistry. The mild reaction conditions, being unaffected by the presence of water, tolerating a broad range of functional groups, the low-toxicity and wide commercial availability of arylboronic acids contribute a lot to the versatility of this reaction.[58]

In the presence of tetrakis(triphenylphosphine)palladium(0) and 2 M sodium carbonate solution, the Suzuki-Miyaura cross-coupling reaction was carried out at 80 °C under nitrogen, giving azabiaryl **149** in a yield of 88% (Scheme 1.14).[64]



148 139 149

Scheme 1.14 *Reagents and conditions*: (a) $Pd(PPh_3)_4$, THF, 2 M Na_2CO_3 aq, 80 °C.

A general catalytic cycle for the Suzuki-Miyaura cross-coupling reaction encompasses four key steps, oxidative addition, ligand exchange, transmetallation and reductive elimination.[58] The mechanism for the cross-coupling reaction is depicted in Figure 1.25.

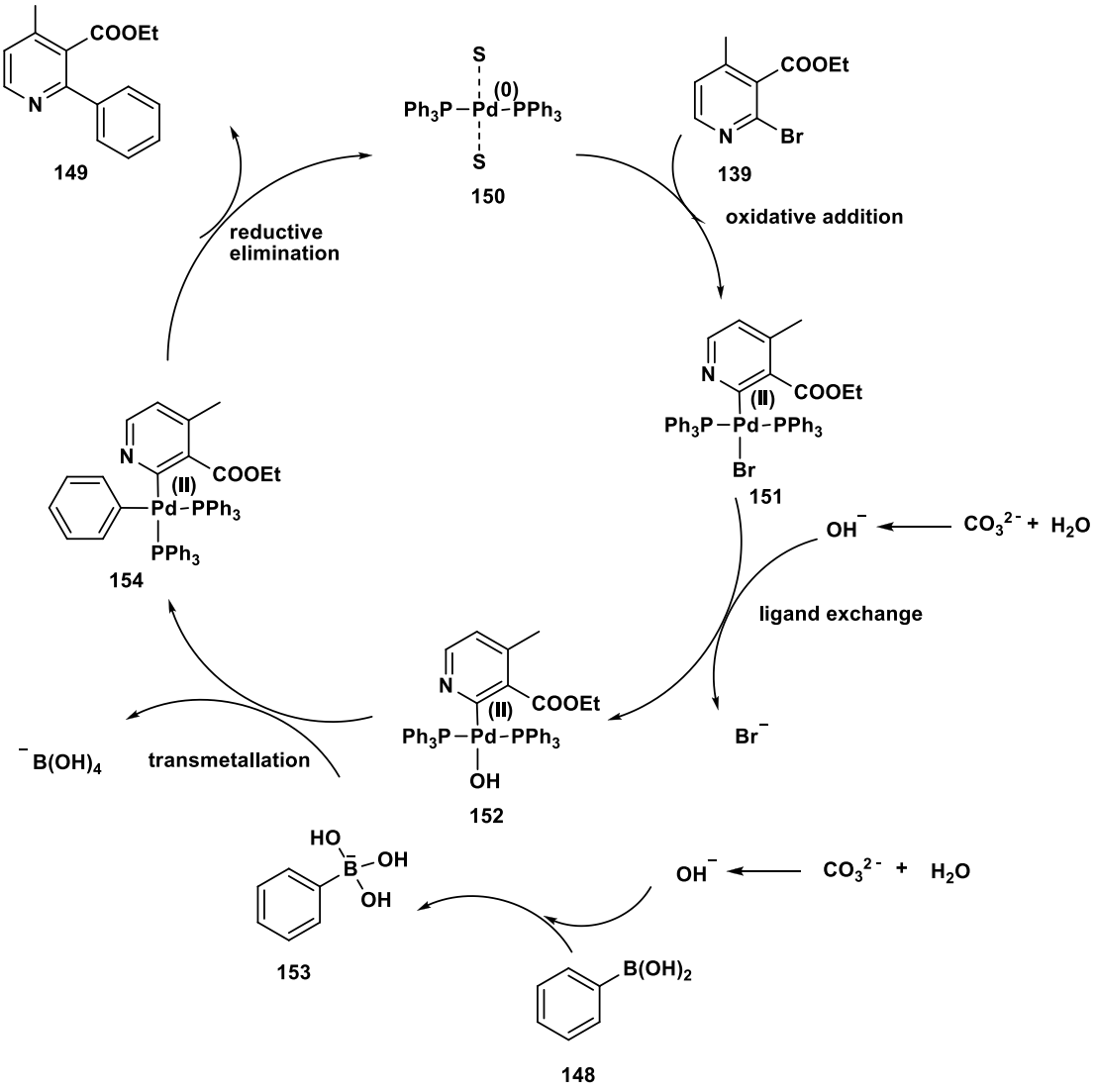


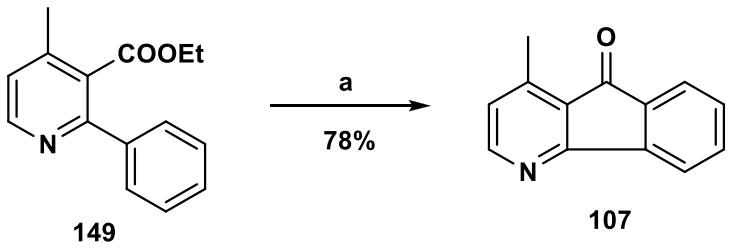
Figure 1.25 Mechanism for the Suzuki cross-coupling.

The oxidative addition of the decorated nicotinate **139** with the palladium(0) reagent **150** forms a σ-aryl-Pd(II)-Br species **151**.[58] The base present in the reaction mixture serves a dual role in the catalytic cycle. The boron atom can be quaternised to form the reactive hydroxyboronate anion **153**. The nucleophilicity of the aryl group in the boronic acid is enhanced by the coordination of the negatively charged base. Meanwhile, the bromide present in the σ-aryl-Pd(II)-Br species **151** can be displaced *via* ligand exchange by a hydroxide ion, affording the aryl-Pd(II)-OH species **154**. The palladium-oxygen bond, which consists of a soft Lewis acid and a hard Lewis base, is more reactive than a palladium-bromine bond, accelerating the subsequent

transmetallation.[58] Reductive elimination from organopalladium species **154** yields azabiaryl **149** and palladium(0) reagent **150**, which re-enters the catalytic cycle.

Having obtained the cyclisation precursor **149**, subsequent intramolecular Friedel-Crafts acylation was performed. Polyphosphoric acid, whose pKa is 2.1, serves as a mild reagent in organic reactions.[65] This accounts for the versatility and extensive application of PPA. Compared with aluminium chloride, it is less moisture sensitive and does not generate hydrogen chloride in reactions. In comparison to concentrated sulphuric acid, PPA does not lead to oxidation of organic compounds. Consequently, PPA usually causes fewer side reactions than other acids used for Friedel-Crafts acylations, such as concentrated sulphuric acid or aluminium chloride.[57]

Although it is usually a carboxylic acid that is exploited in the PPA-catalysed intramolecular acylation,[65-67] some examples have shown that esters can also be cyclised as readily as the acids from which they are derived.[68,69] By heating in polyphosphoric acid,[69] azabiaryl **149** was smoothly cyclised to the fused tricyclic alkaloid analogue **107** in 78% yield (Scheme 1.15).



149 **107**

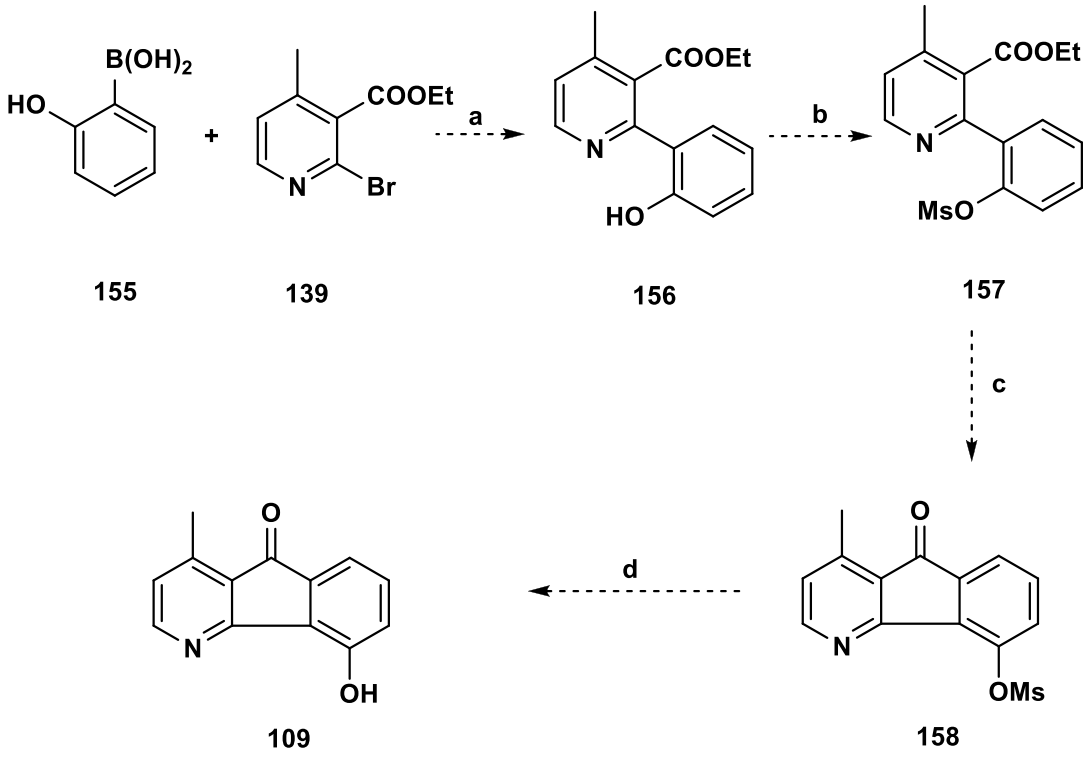
Scheme 1.15 *Reagents and conditions*: (a) PPA, 140 °C.

The successful preparation of the simplified analogue **107** demonstrates that the synthetic route is suitable for the preparation of this class of azafluorenones.

1.2.4 Studies towards the Synthesis of Natural Product 37 and its Analogue 109

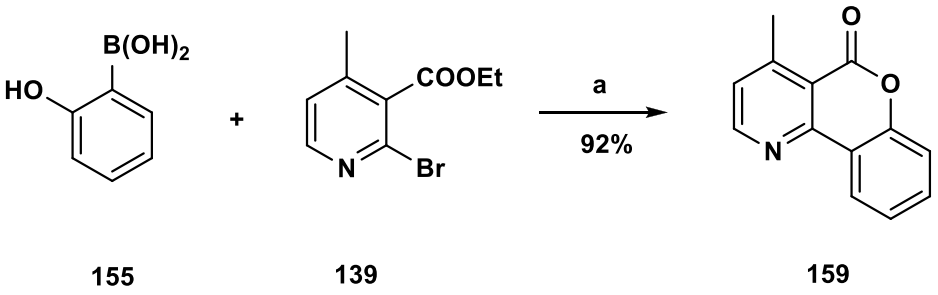
1.2.4.1 Synthesis of Analogue 109 from Unprotected Arylboronic acid

Prior to the synthesis of natural product **37**, the preparation of related analogue **109** was investigated since 2-hydroxy-3-methoxyphenylboronic acid, the starting material for alkaloid **37**, is of high cost (Scheme 1.16).



Scheme 1.16 *Reagents and conditions*: (a) $Pd(PPh_3)_4$, THF, 2 M Na_2CO_3 aq, 80 °C; (b) MsCl, Et_3N, CH_2Cl_2, rt; (c) PPA, 140 °C; (d) NaOH aq, MeOH, 60 °C.

Nicotinate **139** and 2-hydroxyphenylboronic acid **155** were subjected to the previously employed cross-coupling conditions. However, 4-methylpyrido[3,2-*c*]coumarin **159** was formed in a yield of 92%, instead of the desired coupled product (Scheme 1.17).



Scheme 1.17 *Reagents and conditions*: (a) $Pd(PPh_3)_4$, THF, 2 M Na_2CO_3 aq, 80 °C.

Given the ester functional group in the nicotinate **139** and the free hydroxyl group in the arylboronic acid **155**, the formation of pyrido[3,2-*c*]coumarin **159** is predicted to have arisen from a base-catalysed lactonisation after the initial cross-coupling. The proposed mechanism of this conversion is shown in Figure 1.26.

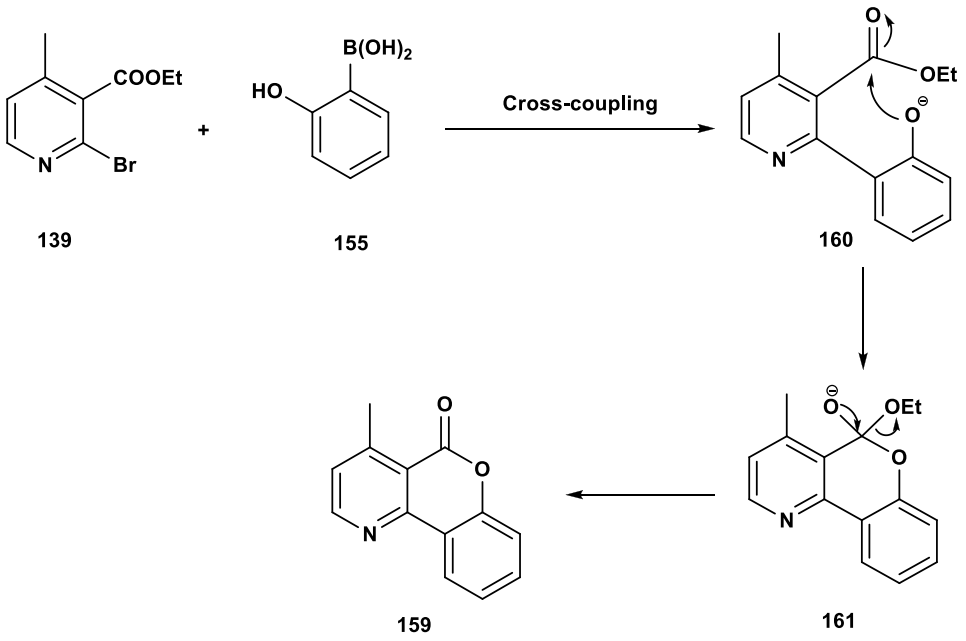
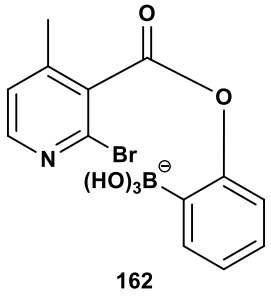


Figure 1.26 Proposed mechanism of the one-pot synthesis of pyrido[3,2-*c*]coumarin.

Initially, the Suzuki-Miyaura cross-coupling is achieved under the palladium(0)-catalysed conditions, yielding azabiaryl **160**. Under the basic condition, the phenol hydroxyl group is deprotonated to form the phenolate anion, whose nucleophilicity is much greater than the phenol. Intramolecular nucleophilic attack of the phenolate anion to the ester group generates tetrahedral intermediate **161**, which

collapses with the loss of ethoxide, affording the tricyclic lactone **159**.

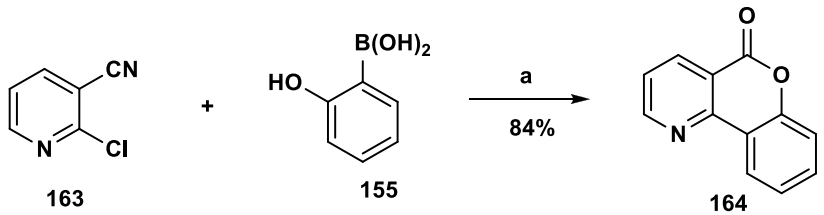
An alternative possibility is that the base-catalysed esterification takes place first, followed by the intramolecular Suzuki-Miyaura cross-coupling. However, there would be a significant steric hindrance in intermediate **162** (Figure 1.27) for the subsequent oxidative addition, which would retard the formation of the σ-aryl-Pd(II)-Br species. Consequently, we believe that the cross-coupling occurs prior to esterification.



162

Figure 1.27 Postulated intermediate obtained from the esterification.

Since nitriles are significantly less reactive than the corresponding esters under basic conditions,[70] it was planned to utilise nitrile substrate **163** to replace ester **139** in the Suzuki reaction with 2-hydroxyphenylboronic acid to construct the azabiaryl. By this means, the undesired lactonisation would be precluded. Nevertheless, when 2-chloronicotinonitrile was subjected to the cross-coupling conditions for chloro-substrates,[71] pyrido[3,2-*c*]coumarin **164** was still afforded in 84% yield (Scheme 1.18).



163 155 164

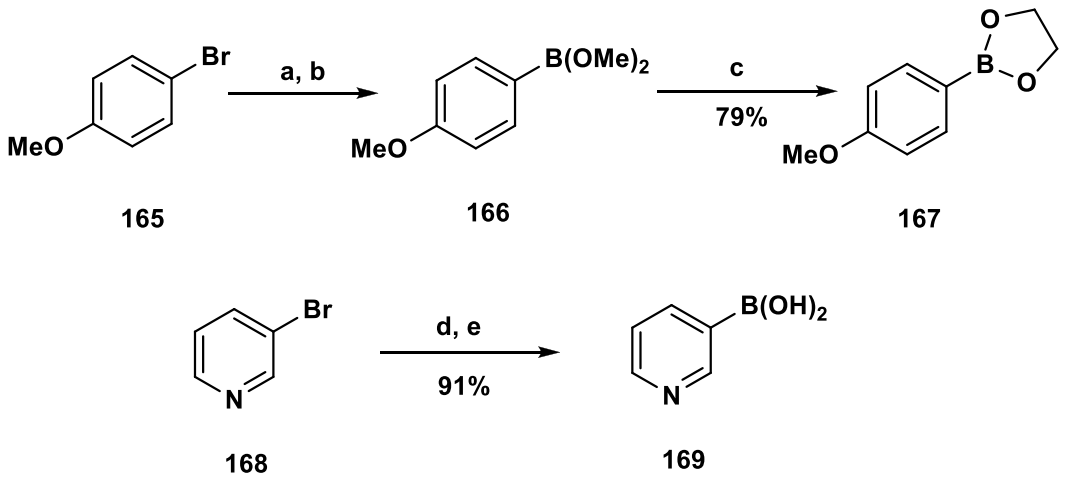
Scheme 1.18 *Reagents and conditions*: (a) $Pd(PPh_3)_4$, DME/H_2O, 2 M K_2CO_3 aq, 80 °C.

The lactonisation of nitriles under these conditions has not been reported in the

literature. Hence, it would be of value to explore this novel method for the formation of pyrido[3,2-*c*]coumarins. In addition, these observations suggest that the protection of the *ortho*-hydroxyl group in the arylboronic acid is required prior to cross-coupling for the synthesis of alkaloid **37** and related analogue **109**.

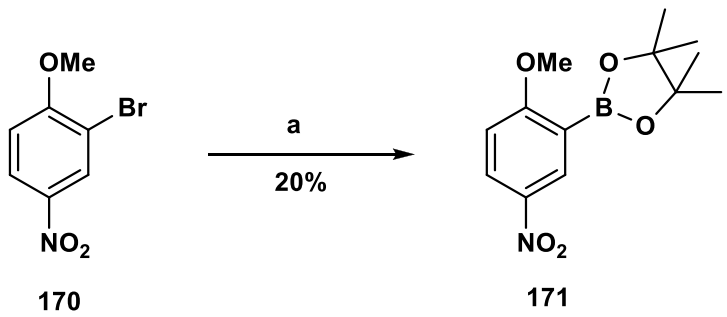
1.2.4.2 Preparation of the Arylboronic Ester for Alkaloid 37

The preceding work has shown that only arylboronic acids with the *ortho*-hydroxyl group protected can afford the desired cross-coupling product for the synthesis of natural product **37**. Since 2-hydroxy-3-methoxyphenylboronic acid, the starting material for alkaloid **37**, is of high cost, it was planned to prepare the requisite protected arylboronic acid from the corresponding precursor 2-bromo-6-methoxyphenol. However, due to the high polarity and amphiphilicity of arylboronic acids, their isolation and purification is usually difficult. As an alternative to arylboronic acids, arylboronic esters also perform efficiently in palladium-catalysed Suzuki-Miyaura cross-coupling reactions.[59] The loss of hydrogen bond donor capability renders boronic esters less polar, dissolvable in apolar solvents and are easier to purify.[59] Commonly used methods for the preparation of arylboronic acids or arylboronic esters involve transmetallation of Grignard reagents or aryllithium with trialkyl boronic esters (Scheme 1.19).[72,73] However, these methods generally utilise strong basic reagents and are incompatible with susceptible functional groups, such as ester, sulfonate, aldehyde and ketone groups.[62]



Scheme 1.19 *Reagents and conditions*: (a) Mg, THF, reflux; (b) $B(OMe)_3$, -78 °C; (c) $HOCH_2CH_2OH$, toluene, reflux; (d) *n*-BuLi, $B(O^iPr)_3$, -40 °C; (e) 2 N HCl aq, -20 °C.

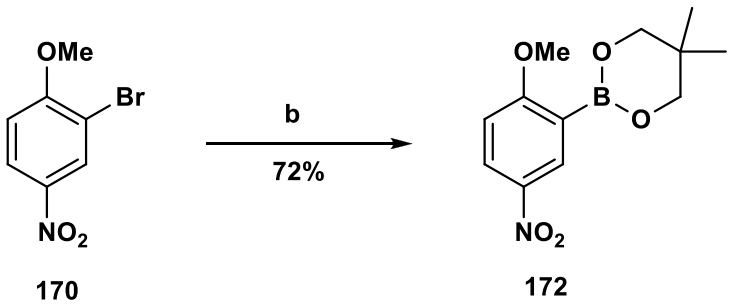
The palladium-catalysed Miyaura borylation, involving the cross-coupling between aryl halides and bis(pinacolato)diboron, possesses higher functional-group compatibility and has been widely used as a viable alternative for the synthesis of arylboronic esters.[74] Although a range of boronic esters have been prepared by this method, borylations of *ortho*-substituted aryl halides have been reported to give low yields, on account of the steric hindrance of the *ortho* substituent (Scheme 1.20).[75]



Scheme 1.20 *Reagents and conditions*: (a) bis(pinacolato)diboron, $Pd(dppf)Cl_2$, KOAc, DMSO, 80 °C.

By employing the less hindered bis(neopentyl glycolato)diboron, the yield of the borylation has been increased (Scheme 1.21).[76] Consequently, it was attempted to apply

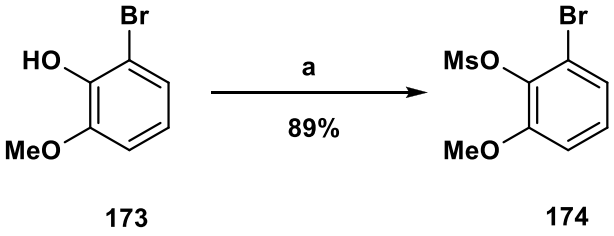
this method to the synthesis of the corresponding arylboronic ester for the target natural product **37**.



170 172

Scheme 1.21 *Reagents and conditions*: (a) bis(neopentyl glycolato)diboron, $Pd(dppf)Cl_2$, KOAc, DMSO, 80 °C.

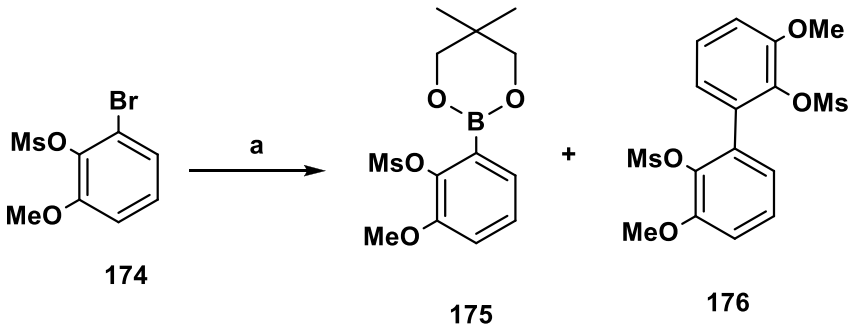
Initially, the methanesulfonyl group was employed to protect the hydroxyl group prior to borylation. In the presence of triethylamine, mesylation of 2-bromo-6-methoxyphenol **173** was fulfilled in a good yield (Scheme 1.22).



173 174

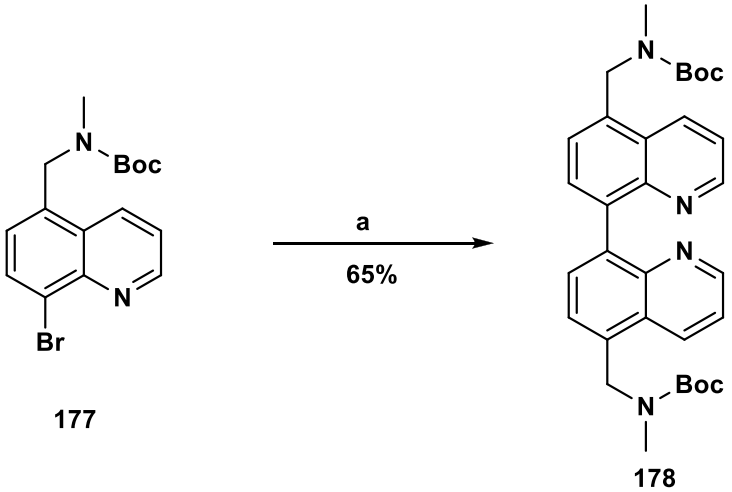
Scheme 1.22 *Reagents and conditions*: (a) MsCl, Et_3N, CH_2Cl_2, rt.

In the presence of [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium ($PdCl_2(dppf)$) and potassium acetate, the borylation of 1-methoxy-2-mesyloxy-3-bromobenzene was performed with bis(neopentyl glycolato)diboron at 80 °C. However, chromatography gave the desired 2-methoxy-6-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)phenyl methanesulfonate **175** in only 7% yield, while 2,2'-methanesulfonyloxy-3,3'-dimethoxybiphenyl **176** was afforded in 36% yield (Scheme 1.23).



Scheme 1.23 *Reagents and conditions*: (a) bis(neopentyl glycolato)diboron, Pd(dppf)Cl_2, KOAc, DMSO, 80 °C.

The formation of biaryl **176** is attributed to the additional Suzuki cross-coupling between arylboronic ester **175** and its precursor **174**. A similar phenomenon has been described in literature with bromide **177** (Scheme 1.24).[77]



Scheme 1.24 *Reagents and conditions*: (a) bis(pinacolato)diboron, Pd(dppf)Cl_2, KOAc, DMSO, 80 °C.

Based on the catalytic cycle of the Miyaura borylation,[74] we can elucidate the formation of boronic ester **175** (Figure 1.28).

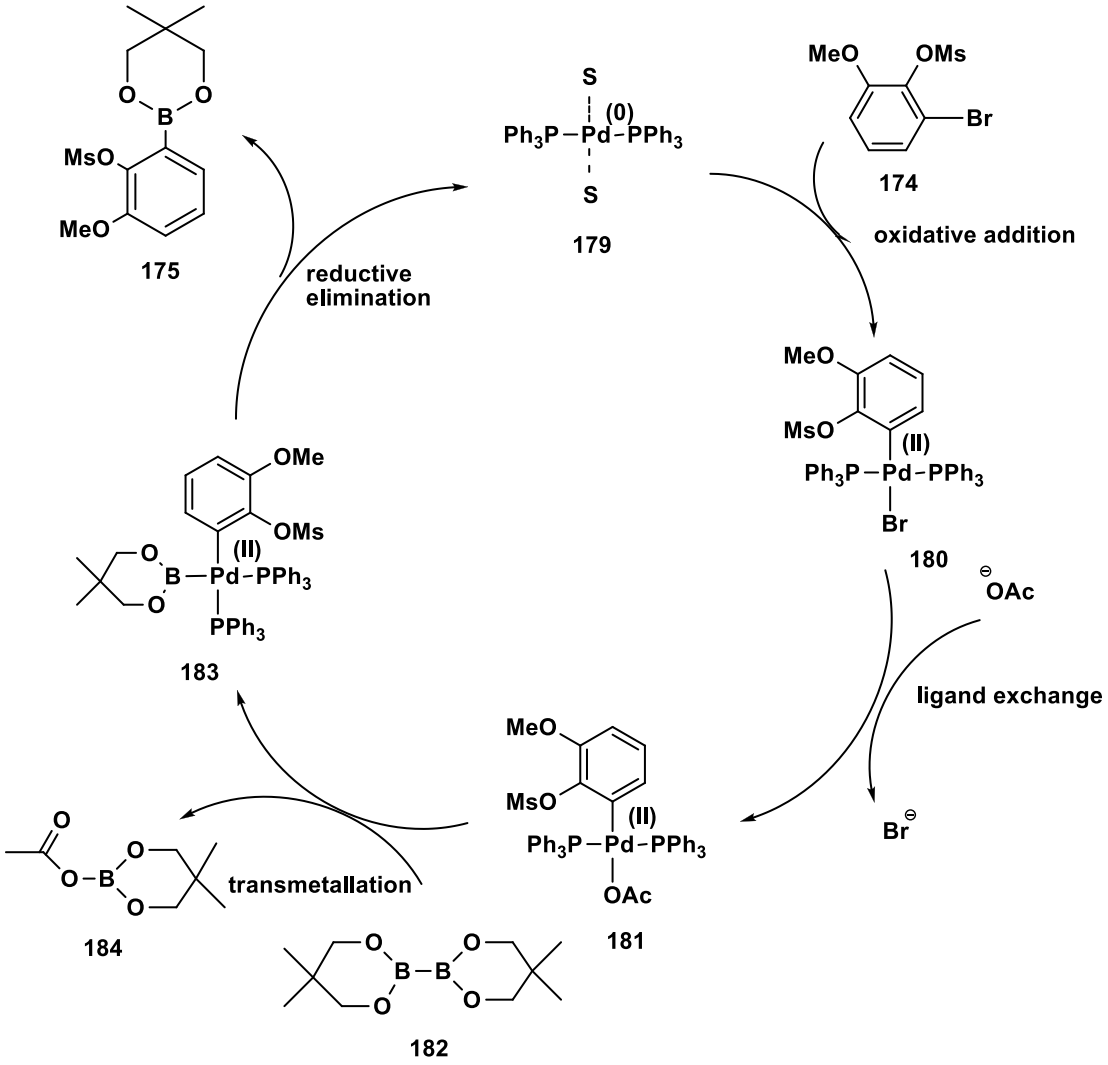


Figure 1.28 Proposed mechanism for the borylation of **174**.

Similar to Suzuki cross-coupling reaction, the palladium-catalysed Miyaura borylation involves oxidative addition, ligand exchange, transmetallation and reductive elimination. The ligand exchange after oxidative addition accelerates the subsequent transmetallation step. It is because that σ-aryl-Pd(II)-OAc species **181**, containing a palladium-oxygen bond composed of a soft Lewis acid and a hard Lewis base, is more reactive than σ-aryl-Pd(II)-Br **180** with a palladium-bromine bond. The palladium (II) complex **183** is produced *via* the following transmetallation. After the reductive elimination, the desired borylated product **175** is generated, with the palladium (0) complex **179** re-entering the catalytic cycle.

However, transmetallation could also occur between σ-aryl-Pd(II)-OAc species **181** and the quaternised arylboronic ester **185** produced from the borylation, initiating the further Suzuki cross-coupling cycle. Hence, biaryl product **176** would be afforded by the subsequent reductive elimination, with palladium (0) complex **179** being regenerated again. The integrated catalytic cycle for both processes is depicted in Figure 1.29.

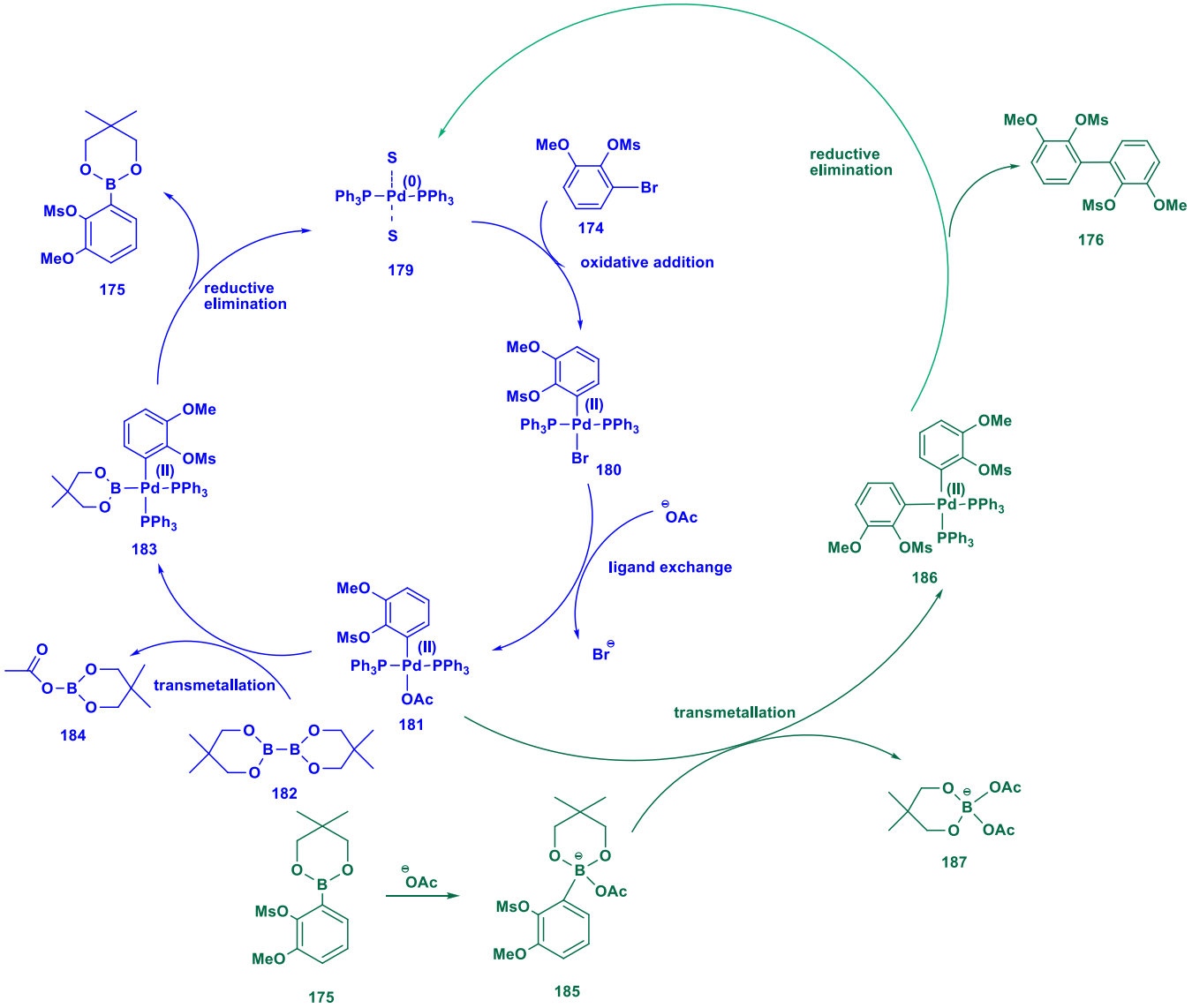
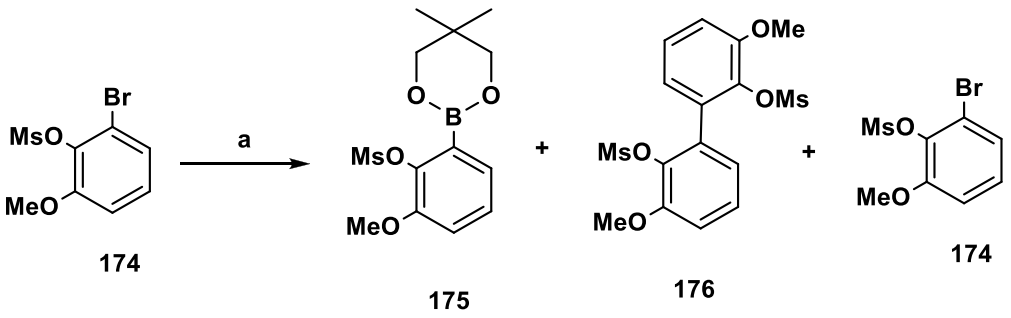


Figure 1.29 Integrated catalytic cycle for the borylation and formation of the biaryl.

To reduce the undesired side cross-coupling reaction, an attempted optimisation of the

borylation was conducted (Table 1.2). We envisioned that if a larger quantity of the borylating reagent was utilised with the reaction temperature raised, the borylation would be promoted and completed in a shorter time. In this case, to reduce the reaction time might make more boronic ester not participate in the further Suzuki cross-coupling. Thus, the borylation would probably become the major reaction within the shortened time. Moreover, the excess borylating reagent would compete with the quaternised aryl boronate **185** in the transmetallation steps, giving rise to a decrease of the Suzuki cross-coupling.



Scheme 1.25 *Reagents and conditions*: (a) bis(neopentyl glycolato)diboron, Pd(dppf)Cl_2, KOAc, DMSO, T °C.

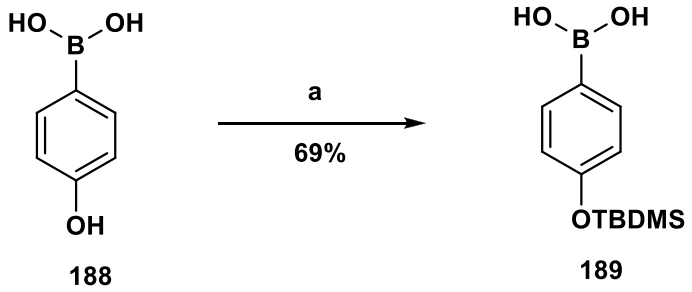
Entry	Bis(neopentyl glycolato)-diboron	KOAc	t (h)	T (°C)	Yield of 175 (%)	Yield of 176 (%)	Yield of 174 (%)
1	1.2 equiv.	3 equiv.	12	80	7	36	0
2	5 equiv.	3 equiv.	6	100	13	33	0
3	5 equiv.	3 equiv.	4	100	11	27	19
4	5 equiv.	1.5 equiv.	5	100	10	21	28

Table 1.2 Attempted optimisation of the borylation.

Borylation with 5 equivalents of bis(neopentyl glycolato)diboron at 100°C for 6 hours did increase the arylboronic ester and reduce the biaryl product (Entry 2). To further decrease the undesired Suzuki reaction, a shorter reaction time was investigated, resulting in 19% unreacted starting material after 4 hours (Entry 3). These observations indicate that it might take 4-6 hours to accomplish the borylation. Based upon the catalytic cycle, a smaller quantity of potassium acetate might decrease the formation of the quaternised aryl boronate **185**, which would also lead to a reduction of the cross-coupled product. Unfortunately, no improvement in borylation was obtained when 1.5 equivalents of potassium acetate were used (Entry 4). This result indicates that decreasing the base might not only reduce the generation of the quaternised aryl boronate **185**, but also retard the desired ligand exchange for the borylation. Given the critical role played by the base in the catalytic cycle, employing alternative bases would be a rational approach to the improvement of borylation.

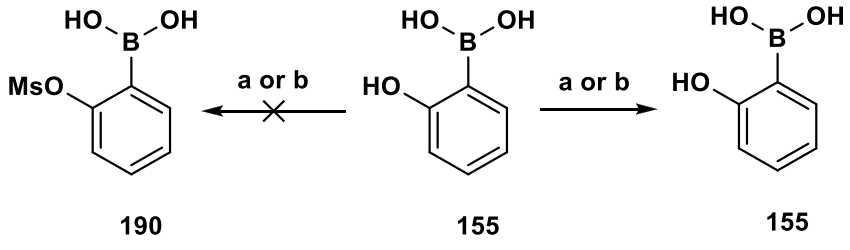
1.2.4.3 Selective Mesylation of 2-Hydroxyphenylboronic Acid

Since the borylation of the aryl bromide had proved unsatisfactory, we had to use the corresponding arylboronic acid instead. A crucial problem is how to protect the *ortho*-hydroxyl group of 2-hydroxyphenylboronic acid **155** prior to the Suzuki cross-coupling reaction. Lautens described the selective protection of the *para*-hydroxyl group of 4-hydroxyphenylboronic acid with *tert*-butyldimethylsilyl (TBDMS) group (Scheme 1.26).[78] This indicates that the phenol hydroxyl group possesses a higher nucleophilicity than the boronic acid hydroxyl groups. Owing to the low stability of *tert*-butyldimethylsilyl ether under acidic conditions,[62] TBDMS cannot be utilised as a protecting group for the synthesis of azafluorenones **37** and **109**. Based on the difference in reactivity, we hoped to selectively protect the *ortho*-hydroxyl group in 2-hydroxyphenylboronic acid with the methanesulfonyl group.



Scheme 1.26 *Reagents and conditions*: (a) TBDMSCl, imidazole, DMF, rt.

Due to the poor solubility of arylboronic acid in dichloromethane, pyridine was employed as a solvent, which would also serve as the auxiliary base absorbing the acid produced in the mesylation. To ensure the boronic acid hydroxyl groups would not be mesylated, a model reaction with phenylboronic acid as the substrate was attempted. No mesylated product was detected after 10 hours, validating the inertness of boronic acid hydroxyl groups towards mesylation. The selective mesylation of 2-hydroxyphenylboronic acid was carried out using methanesulfonyl chloride in pyridine (Scheme 1.27). However, no mesylated product was detected by TLC after 12 hours at room temperature, with a complete recovery of the starting material **155**. Given its accelerating effect on acylations, 4-dimethylaminopyridine (DMAP) was also employed for the mesylation. Unfortunately, this also failed to afford the desired mesylated product.



Scheme 1.27 *Reagents and conditions*: (a) MsCl, Py, rt; (b) MsCl, DMAP, Py, rt.

Due to the presence of the *ortho*-hydroxyl group, 2-hydroxyphenylboronic acid is prone

to form intramolecular hydrogen bonds (Figure 1.30). By virtue of the valence electron deficiency of boron, boronic acids can act as mild Lewis acids coordinating basic groups. Consequently, there is still an intramolecular hydrogen bond even in the ionised molecule (Figure 1.30). The stable six-membered ring renders this binding extraordinarily strong. We believe it is this strong intramolecular hydrogen bond that precluded the mesylation of the *ortho*-hydroxyl group.

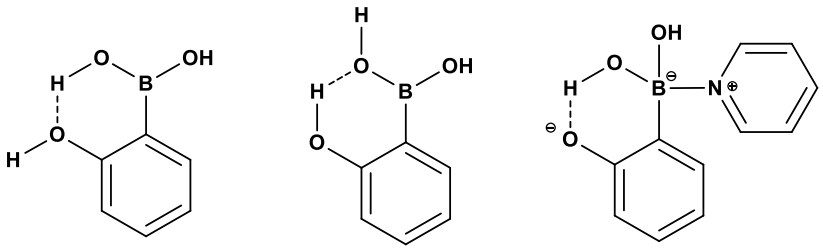
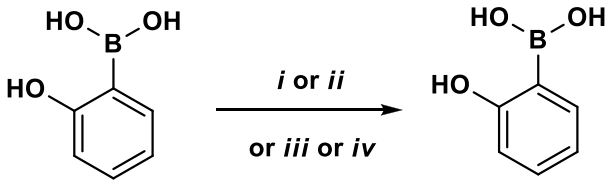


Figure 1.30 Intramolecular hydrogen bonds in **155** and its ionised counterpart.

To overcome the intramolecular hydrogen bonds and promote the mesylation, the reaction temperature was raised to 60 °C (Table 1.3, Condition *i*), whereas no product formation was detected. Triethylamine is frequently exploited as an alternative for pyridine in acylations. Hence, triethylamine was employed instead in the mesylation to serve as an auxiliary base absorbing the acid produced (Condition *ii*). However, this mesylation failed again. Since treatment with *p*-toluenesulfonyl chloride in the presence of potassium carbonate under reflux in acetone is a widely utilised condition for the tosylation of phenols,[79] attempted mesylation under conditions *iii* and *iv* were conducted accordingly. Unfortunately, neither of the two attempts provided the desired mesylated product.

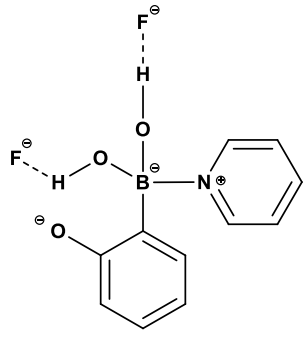


155 155

Conditions	Catalytic Base	Solvent	Temperature (°C)
i	DMAP	Pyridine	60
ii	DMAP, Et_3N	THF	55
iii	K_2CO_3	Acetone	60 (reflux)
iv	K_2CO_3, DMAP	Acetone	60 (reflux)

Table 1.3 Attempted mesylation under different conditions.

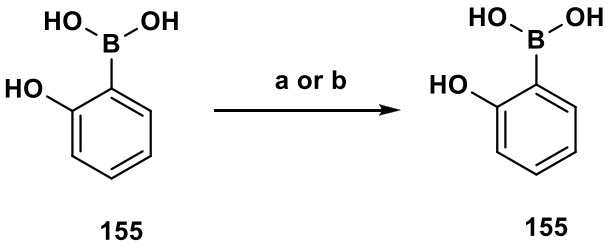
Since these attempts failed to bring about improvements in the mesylation, it was planned to utilise some reagents to weaken the intramolecular hydrogen bonds in arylboronic acid **155**. The negative charge and small size of the fluoride anion enable it to be a good hydrogen bonding acceptor.[80] Therefore, it was envisaged that the addition of fluoride anion would weaken the inherent intramolecular hydrogen bonds in 2-hydroxyphenylboronic acid by forming intermolecular hydrogen bonds between the hydroxyl group and fluoride anion and thus facilitate mesylation (Figure 1.31).



194

Figure 1.31 Attempt at weakening the intramolecular hydrogen bonds in ionised **155**.

Nevertheless, the attempts at mesylation with tetrabutylammonium fluoride (TBAF) as the source of fluoride anion also failed to afford the desired product, with only 2-hydroxyphenylboronic acid recycled (Scheme 1.28).



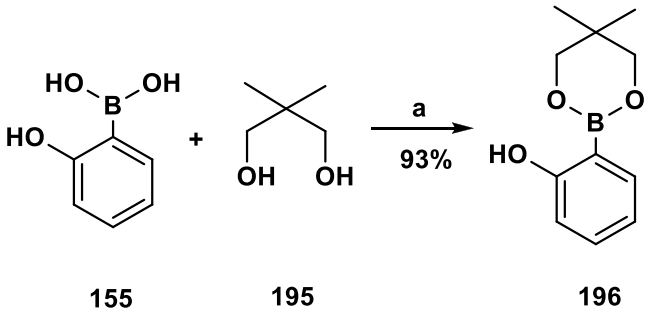
155 155

Scheme 1.28 *Reagents and conditions*: (a) MsCl, DMAP, TBAF, Py, 60 °C; (b) MsCl, DMAP, TBAF, Et_3N, THF, 55 °C.

These attempts suggest that the inherent intramolecular hydrogen bond in 2-hydroxyphenylboronic acid is too strong to overcome. Accordingly, the strategy was modified to protect the boronic acid hydroxyl groups before the mesylation. Under basic conditions, there would be no such intramolecular hydrogen bonds retarding the mesylation of the corresponding arylboronic ester.

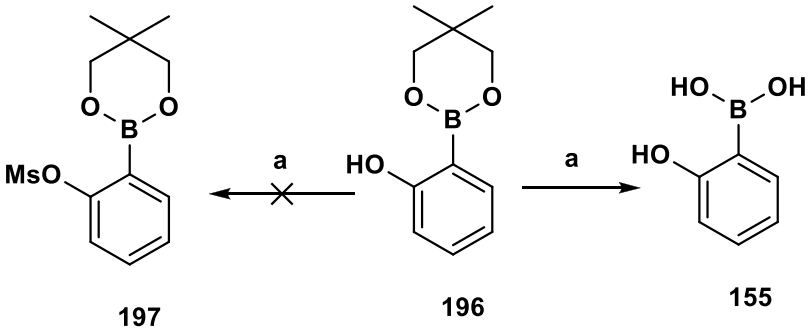
1.2.4.4 Synthesis of Azafluorenone 109 from Mesylated Arylboronic Ester

The protection of the boronic acid hydroxyl groups was accomplished through the treatment with 2,2-dimethylpropane-1,3-diol, affording the arylboronic ester **196** in an excellent yield (Scheme 1.29). This conversion was achieved *via* a nucleophilic addition towards the electron-deficient boron centre, followed by the nucleophilic substitution.



Scheme 1.29 *Reagents and conditions*: (a) toluene, Dean-Stark apparatus, reflux.

Mesylation of the arylboronic ester **196** was conducted using methanesulfonyl chloride. Unexpectedly, after 6 hours at room temperature, the product obtained was deprotected 2-hydroxyphenylboronic acid **155**, instead of the desired mesylated product **197** (Scheme 1.30).

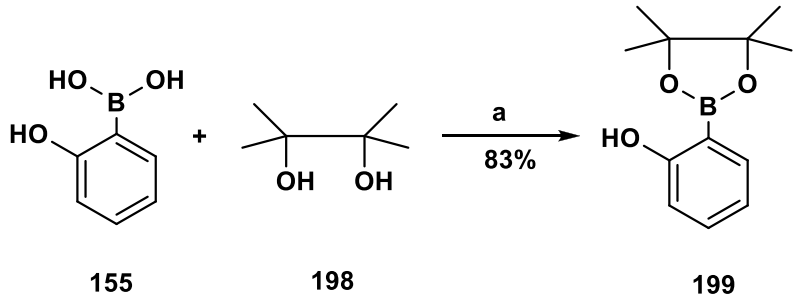


Scheme 1.30 *Reagents and conditions*: (a) MsCl, Et_3N, CH_2Cl_2, rt.

It has been reported that the arylboronic esters protected by 2,2-dimethylpropane-1,3-diol are unstable.[81] Boronic ester **196** decomposes under the mesylation conditions to afford 2-hydroxyphenylboronic acid which can not be mesylated owing to the strong intramolecular hydrogen bonds.

Pinacol is another diol commonly used for the protection of arylboronic acids.[59] With a larger and sterically bulky group, the hydrolysis of the boronic ester would be less

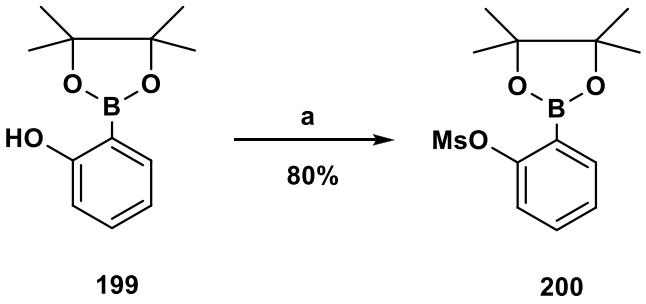
facile. Therefore, pinacol was employed to protect the arylboronic acid hydroxyl groups. With anhydrous magnesium sulphate and molecular sieve being used to remove the water generated, the conversion was achieved in a yield of 83% (Scheme 1.31).



155 198 199

Scheme 1.31 *Reagents and conditions*: (a) $MgSO_4$, molecular sieve, CH_2Cl_2, rt.

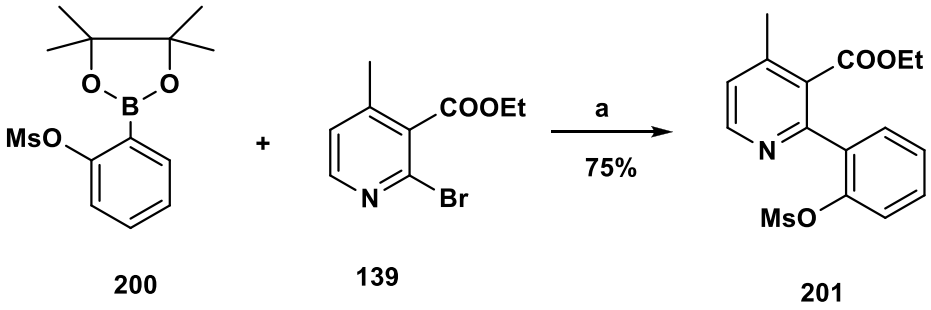
The mesylation of the arylboronic ester **199** was performed with methanesulfonyl chloride in the presence of triethylamine, with the anticipated mesylated product **200** being obtained in 80% yield (Scheme 1.32).



199 200

Scheme 1.32 *Reagents and conditions*: (a) MsCl, Et_3N, CH_2Cl_2, rt.

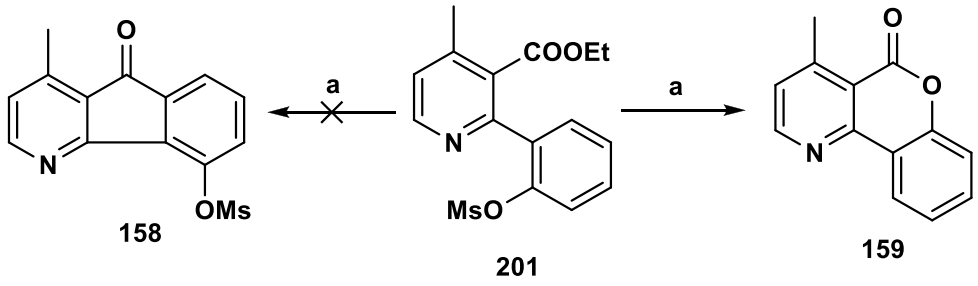
In the presence of tetrakis(triphenylphosphine)palladium(0) and 2 M sodium carbonate solution, the cross-coupling between the mesylated product **200** and nicotinate **139** was achieved in 75% yield (Scheme 1.33). This result demonstrates that our method developed for the cross-coupling of arylboronic acid substrates is also efficient for the arylboronic ester substrates.



200 139 201

Scheme 1.33 *Reagents and conditions*: (a) $Pd(PPh_3)_4$, THF, 2 M Na_2CO_3 aq, 80 °C.

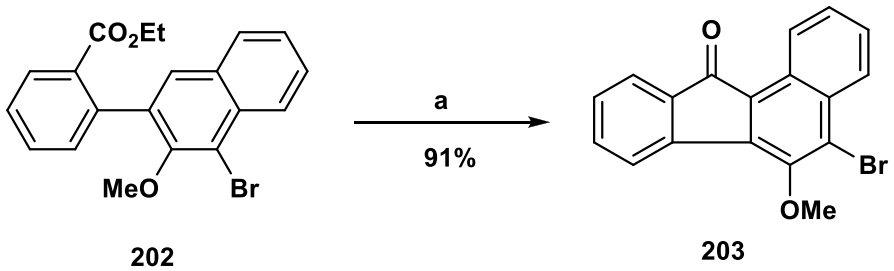
The previous model reactions had verified the stability of methanesulfonate esters under the PPA-catalysed cyclisation conditions. Accordingly, azabiaryl **201** was subjected to the intramolecular acylation catalysed by PPA. However, pyrido[3,2-*c*]coumarin derivative **159**, rather than the desired azafluorenone **158**, was formed after 5.5 hours again (Scheme 1.34). This can be speculated to arise from the unexpected hydrolysis of the sulfonate ester followed by lactonisation.



158 201 159

Scheme 1.34 *Reagents and conditions*: (a) PPA, 130 °C.

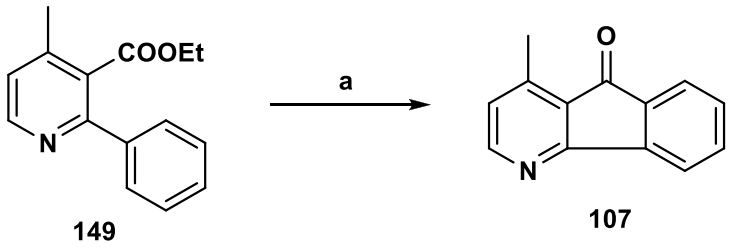
The literature reveals that methanesulfonic acid is another reagent widely applied to acid-catalysed acylations (Scheme 1.35).[82]



202 203

Scheme 1.35 *Reagents and conditions*: (a) MsOH, 65 °C.

Since the hydrolysed product of methanesulfonate ester is just methanesulfonic acid, an excess of methanesulfonic acid present in the reaction mixture would be anticipated to inhibit the equilibrium of the hydrolysis shifting towards the undesired hydrolysed product. Model reactions with **149** as the cyclisation precursor were screened for the suitable alternative cyclisation conditions with methanesulfonic acid for our substrates (Scheme 1.36).



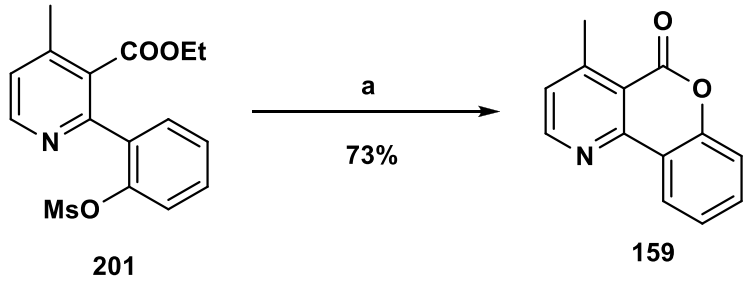
Scheme 1.36 *Reagents and conditions*: (a) MsOH, T °C.

Attempted acylations at 65 °C and 75 °C both failed to yield the desired cyclisation product after 24 hours, with substrate **149** recovered from the reaction mixture. When the reaction temperature was increased to 95 °C, the anticipated azafluorenone **107** was afforded after stirring in methanesulfonic acid for 24 hours, with only a little unreacted substrate **149** recycled. Further increase of the reaction temperature to 100 °C achieved the complete conversion in 24 hours and improved the yield to 71% (Table 1.4).

Entry	Reaction Temperature (°C)	Yield (%)
1	65	0
2	75	0
3	95	62
4	100	71

Table 1.4 Screening cyclisation conditions for the azabiaryls with MsOH.

Having established a procedure for the methanesulfonic acid-catalysed acylation, azabiaryl **201** was subjected to these conditions (Scheme 1.37). However, pyrido[3,2-*c*]coumarin **159** was formed again, instead of the target azafluorenone **158**.



201 159

Scheme 1.37 *Reagents and conditions*: (a) MsOH, 100 °C.

This result can be ascribed to the high stability of the conjugated tricyclic structure of lactone **159** which served as the impetus to make the reaction equilibrium shift towards the cleavage of methanesulfonate ester group, affording pyrido[3,2-*c*]coumarin **159**.

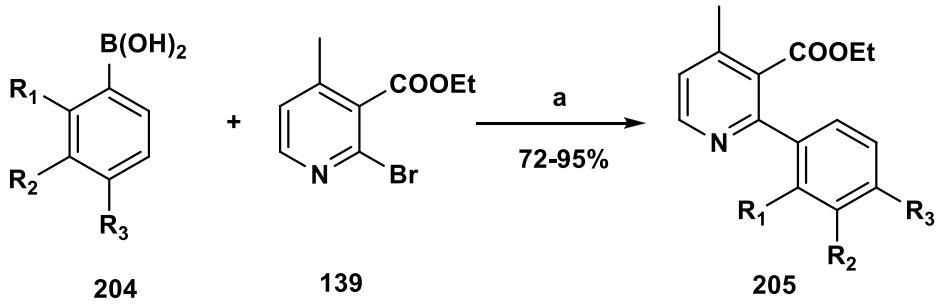
All of these attempts suggest that the synthetic route involving the cross-coupling and PPA-catalysed intramolecular Friedel-Crafts acylation may be inappropriate for the preparation of the natural product **37** and its analogue **109**. Another strategy employing a Grignard reaction to connect the pyridyl and phenyl moieties at β-position followed by an intramolecular Heck reaction to construct the tricyclic ring system might be an alternative approach for the synthesis of alkaloid **37** and its analogue **109**.[52]

1.2.5 Synthesis of Azafluorenone Analogues Modified on the Phenyl Moiety

To explore the structure-activity relationship concerning the phenyl moiety, a series of azafluorenone analogues with modifications on the phenyl ring were prepared utilising the synthetic route for the parent core **107**.

In the presence of palladium reagent and 2 M sodium carbonate solution, Suzuki-Miyaura cross-coupling reactions between nicotinate **139** and a range of

commercially available arylboronic acids were achieved at 80 °C under nitrogen. The azabiaryls were afforded in moderate to excellent yields (Scheme 1.38).



Scheme 1.38 *Reagents and conditions*: (a) $Pd(PPh_3)_4$, THF, 2 M Na_2CO_3 aq, 80 °C.

The results for the Suzuki reaction with various boronic acid substrates are summarised in Table 1.5. The cross-couplings were more facile when the boronic acid substrate possessed an electron-donating group (Entry 2 *versus* Entry 1) and were retarded in the case of the boronic acids bearing an electron-withdrawing group (Entry 3 *versus* Entry 1). This can be attributed to the effects these functional groups exerted on the transmetallation step in the cross-coupling cycle (Figure 1.32).

Entry	Boronic Acid	R_1	R_2	R_3	Product	Yield (%)
1	**148**	H	H	H	**149**	88
2	**206**	H	OMe	H	**211**	95
3	**207**	F	H	H	**212**	72
4	**208**	Me	H	H	**213**	91
5	**209**	H	H	F	**214**	78
6	**210**	H	H	Me	**215**	89

Table 1.5 Suzuki cross-couplings of different arylboronic acid substrates.

In the transmetallation step, the aryl group in boronic acid **216** is transferred to the electrophilic palladium complex **152** (Figure 1.32). An electron-donating group enhances the electron density and hence the nucleophilicity of the arylboronic acid, greatly facilitating this nucleophilic transfer and promoting the cross-coupling reaction. Conversely, an electron-withdrawing group reduces electron density on the arylboronic acid and retards the transmetallation step.

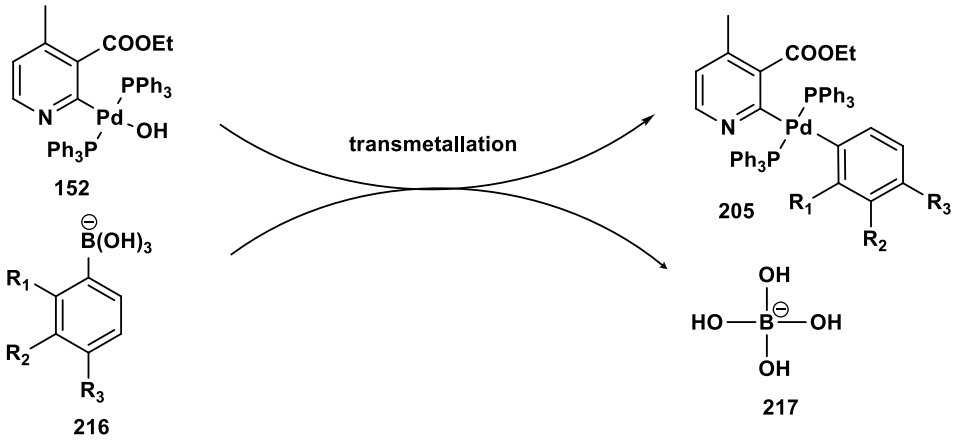
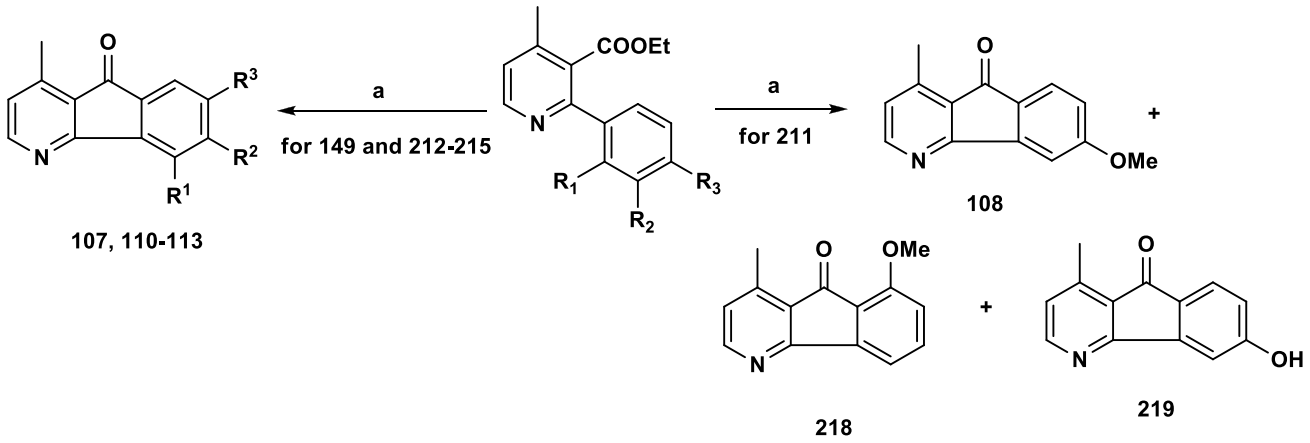


Figure 1.32 Transmetallation in the Suzuki cross-coupling catalytic cycle.

Having obtained the cyclisation precursors, intramolecular acyalations were achieved through treatment of the azabiaryls with polyphosphoric acid at 140 °C (Scheme 1.39). The results for cyclisations are summarised in Table 1.6. In the case of substrate **211** bearing a methoxy group, both 6-methoxy-1-methyl-4-azafluoren-9-one **108** and 8-methoxy-1-methyl-4-azafluoren-9-one **218** were afforded, due to the *ortho*-/*para*-directing effect of the methoxy group. It was noteworthy that 6-hydroxy-1-methyl-4-azafluoren-9-one **219** was also obtained, as a result of the cleavage of the methoxy group under the acidic reaction condition and high temperature employed for the cyclisation.



Scheme 1.39 *Reagents and conditions*: (a) PPA, 140 °C.

Two plausible mechanisms might be responsible for the PPA-catalysed intramolecular cyclisations. One is the pathway *via* an acylium ion intermediate, outlined in Figure 1.33. Protonation of the ethoxyl group in **205** forms a good leaving group ethanol, which is ejected to afford the acylium ion **221**. The aromatic carbon-carbon double bond in **221** serves as a nucleophile to attack the electrophilic acylium ion. Subsequent removal of the proton from the ring reforms the carbon-carbon double bond and restores the aromaticity, affording the target tricyclic azafluorenone.

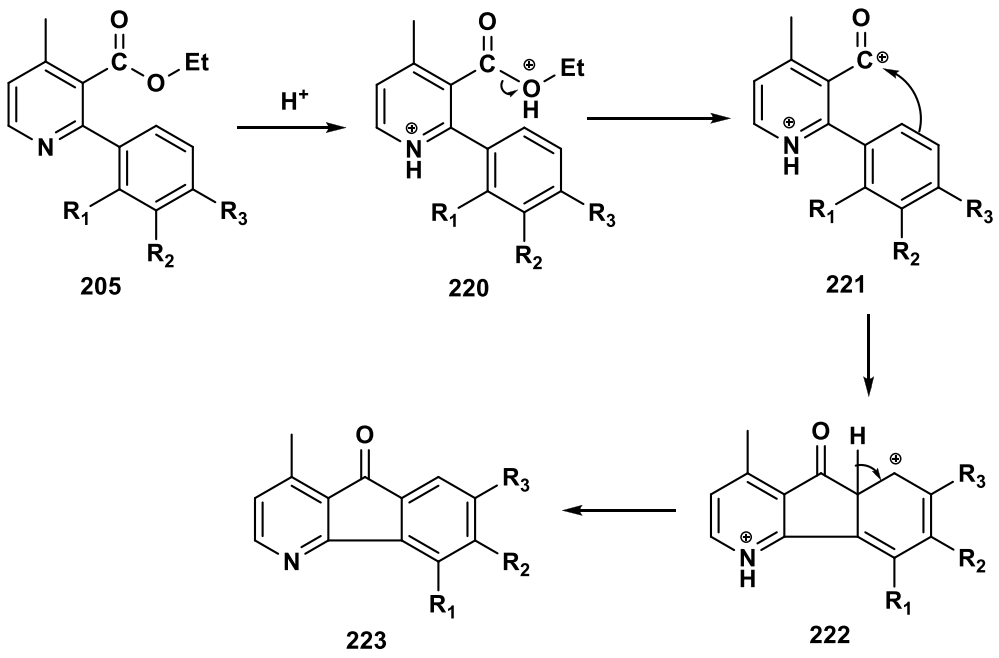


Figure 1.33 Proposed mechanism of cyclisation involving acylium ion.

Alternatively, the PPA-catalysed intramolecular cyclisations might be achieved *via* another mechanism involving a tetrahedral intermediate (Figure 1.34). Protonation of the carbonyl oxygen increases the degree of positive charge at the carbonyl carbon and thus makes it a better electrophile. The nucleophilic attack from the aromatic carbon-carbon double bond results in the formation of the tetrahedral intermediate **224** and constructs the fused tricyclic system simultaneously. The proton transfer from the phenyl to the ethoxyl group gives **225**, which then collapses, with ethanol ejected as the leaving group and forms the oxonium species **226**. Deprotonation of **226** affords the target azafluorenone.

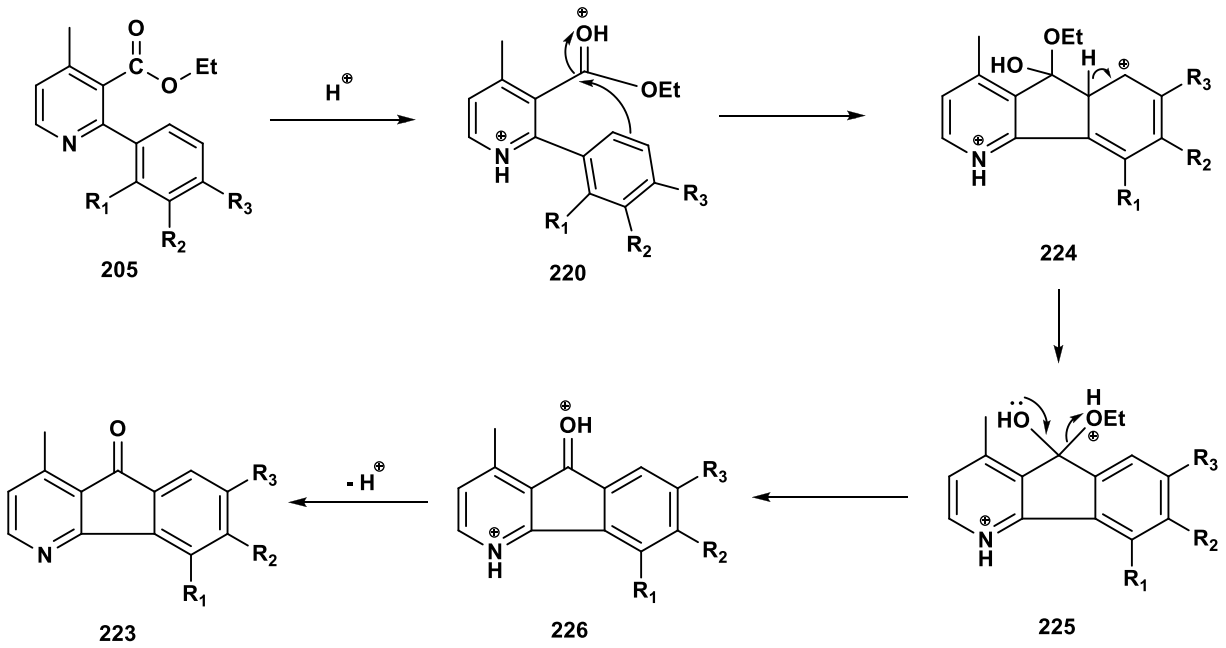
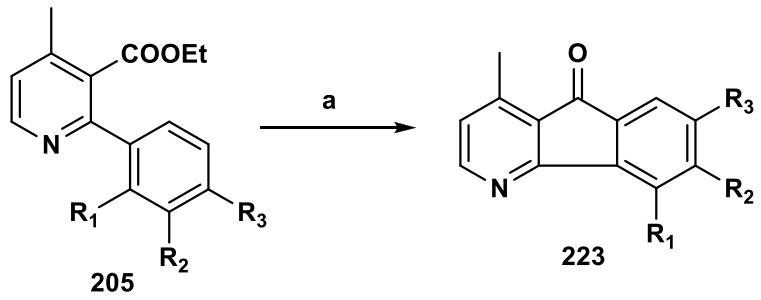


Figure 1.34 Proposed mechanism of cyclisation *via* tetrahedral intermediate.

In terms of electronic and steric effects on the key intermediates, we believe the second mechanism proposal is more reasonable. In the first proposed pathway, the generation of intermediate **221** is the rate-determining step (Figure 1.33). In theory, the positively charged acylium ion can be stabilised by an electron-donating group. However, the acylium ion in **221** is attached to the electron-deficient pyridine ring, which cannot serve as an efficient electron-donating group to stabilise this acylium ion intermediate. Accordingly, the formation of acylium ion **221** is not favoured. In the second mechanism, the formation of the tetrahedral intermediate **224** is the rate-determining step (Figure 1.34). The steric effect is a key factor influencing the efficiency of addition-elimination pathway of esters. When the ester group contains a bulky alkoxy group, such as *tert*-butoxy group, the attack from nucleophiles will be retarded. However, the ethoxy group present in **220** is relatively small, which will not block the nucleophilic attack from the aromatic carbon-carbon double bond to afford the tetrahedral intermediate **224**.

As shown in Table 1.6, the intramolecular acylations of fluorinated azabiaryls are severely retarded (substrates **212** and **214**). Several factors account for the low yields

observed for the cyclisations of the 2'-fluoro- and 4'-fluoro-substrates.



Scheme 1.40 *Reagents and conditions*: (a) PPA, 140 °C.

Substrate	R_1	R_2	R_3	Product	Yield of Product (%)	Yield of Recovered SM (%)
149	H	H	H	**107**	78	0
211	H	OMe	H	**108**, **218**, **219**	Compound **108**, 33	0
					Compound **218**, 28	
					Compound **219**, 24	
212	F	H	H	**110**	31	54
213	Me	H	H	**111**	80	0
214	H	H	F	**112**	26	51
215	H	H	Me	**113**	75	0

Table 1.6 Summary of the PPA-catalysed cyclisations.

Firstly, due to the high electronegativity of fluorine, the electron-donating resonance effect involving the lone pairs of fluorine is insufficient to overcome its strong electron-withdrawing inductive effect on the arene. Consequently, the electrophilic substitution is deactivated by the overall electron-withdrawing effect. Although deactivated, the attack tends to occur at the *ortho* or *para* position, because fluorine can

stabilise the carbocation intermediate derived from *ortho* or *para* attack by resonance. When the electrophilic attack takes place at the *ortho* position, there is an additional canonical structure **229** accessible for the resonance hybrid of the intermediate (Figure 1.35). The stable canonical form **229** contributes most to the intermediate.[83] As a result, the carbocation intermediate generated by the *ortho* attack can be stabilised to some extent, owing to the existence of the additional canonical form **229**. A similar situation arises for *para* substitution.

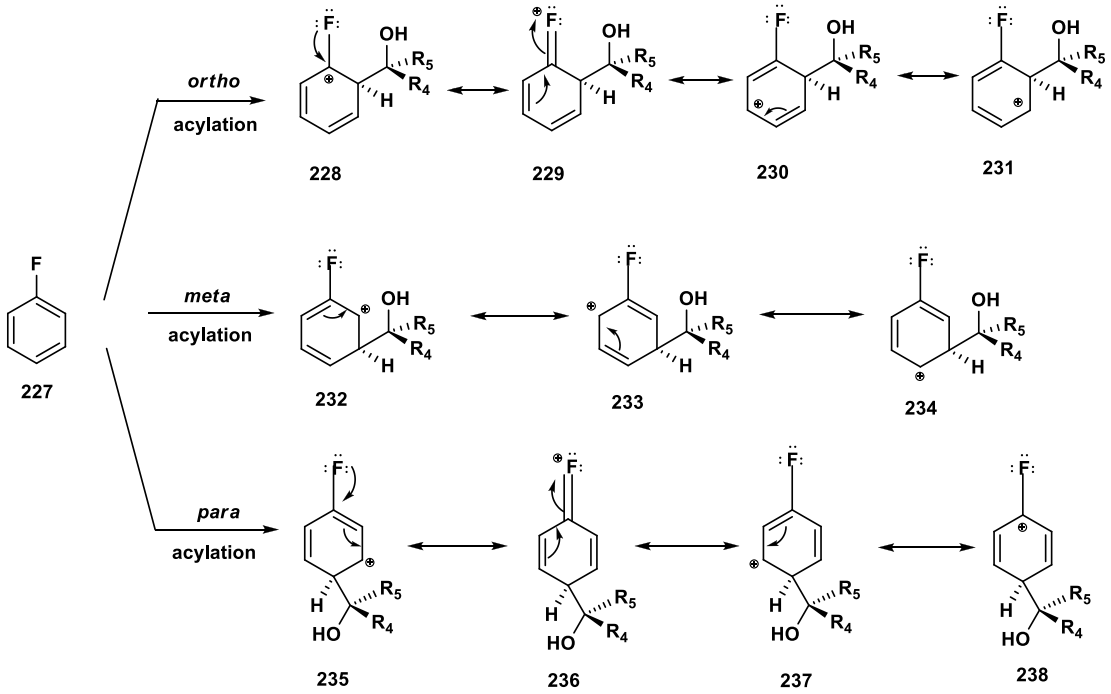


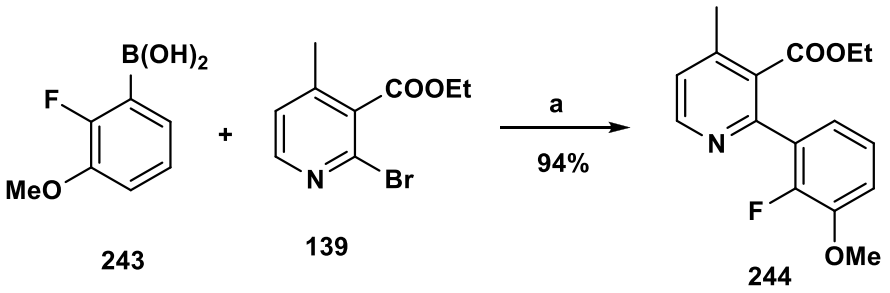
Figure 1.35 Carbocation intermediates in the acylation of fluorinated substrate.

Although the *ortho* or *para* acylation is favoured in terms of the electronic effect, the corresponding product yielded by the *ortho* or *para* attack contains sp^2-hybridised carbons located at the bridgeheads of the bridged ring system, which is of high instability according to Bredt's rule.[84] Consequently, the cyclisation has to take place at the *meta* position of the 2'-fluoro- or 4'-fluoro-substrate to form a planar fused tricyclic structure, although it is the most deactivated way of acylation. These factors gave rise to the low yields for the cyclisation of fluorinated precursors **212** and **214**.

1.2.6 Synthesis of 5-Fluoro-6-methoxy-1-methyl-4-azafluoren-9-one

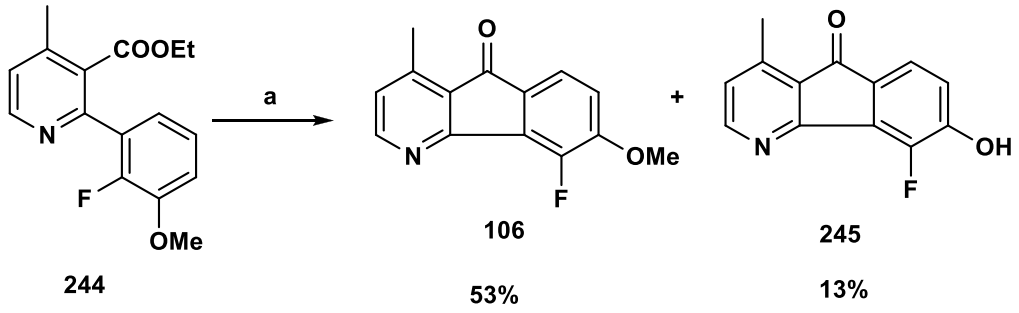
By employing an identical strategy towards the synthesis of azafluorenone modified on the phenyl moiety, the fluorinated analogue of the natural product was prepared using a concise synthetic route.

The Suzuki cross-coupling of nicotinate **139** with arylboronic acid **243** was performed in the presence of tetrakis(triphenylphosphine)palladium(0) and sodium carbonate to provide the cyclisation precursor **243** in an excellent yield (Scheme 1.41).



Scheme 1.41 *Reagents and conditions*: (a) $Pd(PPh_3)_4$, THF, 2 M Na_2CO_3 aq, 80 °C.

The tricyclic core was assembled again by an intramolecular cyclisation of **244** promoted by polyphosphoric acid at 130 °C. Azafluorenones **106** and **245** were both obtained from this intramolecular acylation, in 53% and 13% yield, respectively (Scheme 1.42).



Scheme 1.42 *Reagents and conditions*: (a) PPA, 130 °C.

1.2.7 Synthesis of Azafluorenone Analogues Modified on the Pyridyl Moiety

To investigate the structure-activity relationship regarding the pyridyl moiety, a range of azafluorenone analogues with modifications on the pyridyl ring were synthesised based on the bioisostere **106**.

The preparations of 3-chlorinated azafluorenone **115** and 3-methoxylated azafluorenone **116** were commenced from *N*-oxide **246** due to the higher reactivity of this substrate (Figure 1.37). In the structure of *N*-oxide **246**, the nitrogen is endowed with stronger electron-withdrawing capacity because it's positively charged. As a result, the 3-position is rendered more electron-deficient and more susceptible to nucleophilic attack relative to analogue **106**.

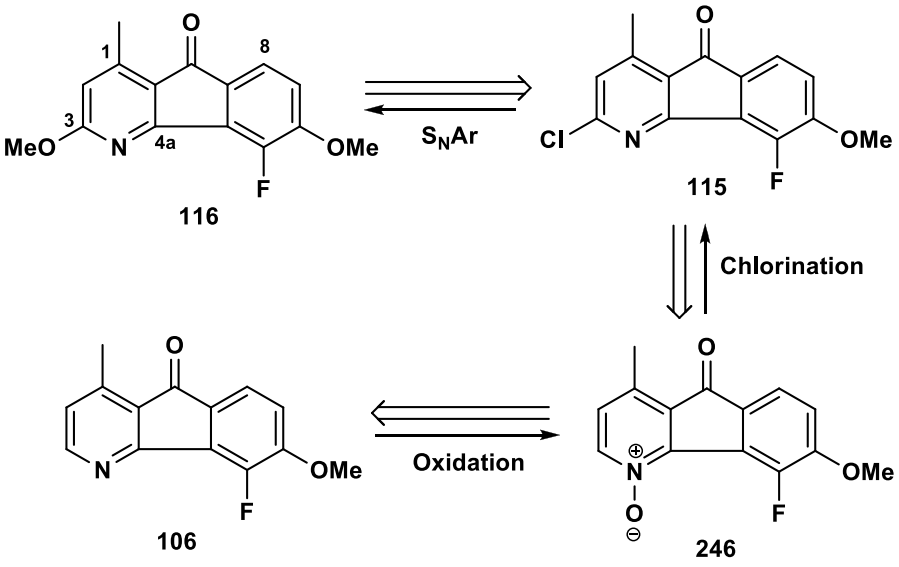
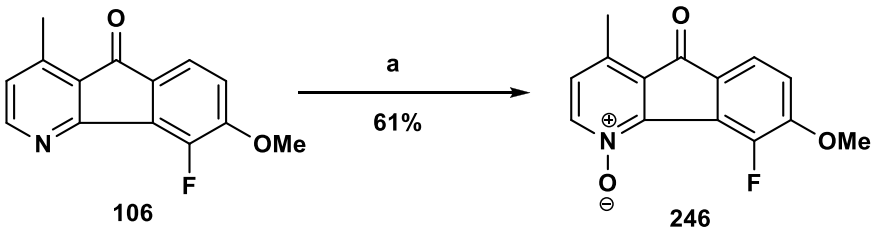


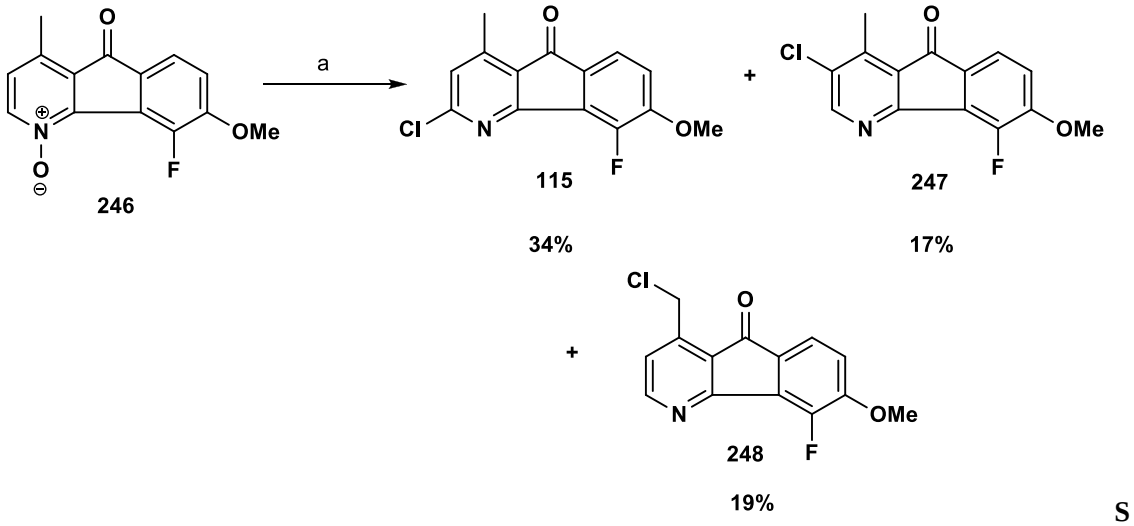
Figure 1.37 Retrosynthetic analysis for azafluorenones **115** and **116**.

The oxidation of **106** was achieved through treatment with 30% hydrogen peroxide aqueous solution in acetic acid at 80 ºC, providing *N*-oxide **246** in 61% yield (Scheme 1.43).



Scheme 1.43 *Reagents and conditions*: (a) 30% H_2O_2 aq, AcOH, 80 °C.

Chlorination of pyridine *N*-oxides through treatment with phosphorus oxychloride[84] not only provided the target azafluorenone **115** in a yield of 34%, but also afforded its isomers **247** and **248** (Scheme 1.44).



Scheme 1.44 *Reagents and conditions*: (a) $POCl_3$, 100 °C.

The generation of **115** is supposed to follow the mechanism shown in Figure 1.38. Nucleophilic attack from the negatively charged oxygen in *N*-oxide **246** to phosphorus oxychloride generates the adduct **250**, which ejects chloride anion as the leaving group to yield intermediate **251**. The chloride anion generated attacks the activated α-position to form adduct **252**. Elimination of dichlorophosphate and proton restores the aromaticity and provides the chlorinated product **115** (Figure 1.38).

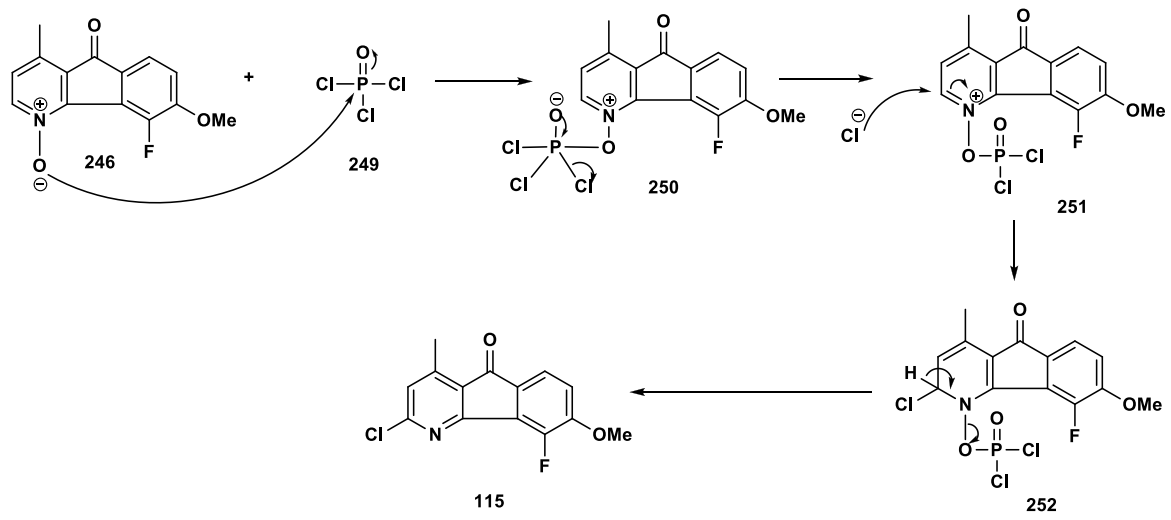


Figure 1.38 Proposed mechanism for the formation of **115**.

The electron-withdrawing effect of the positively charged nitrogen renders the γ-methyl group in **253** susceptible to the loss of a proton to yield intermediate **254** (Figure 1.39). The exocyclic methylene group in **254** is susceptible to nucleophilic attack due to the presence of the good leaving group dichlorophosphate. The chloride anion formed from the previous elimination can attack this methylene group to afford chlorinated product **248**.

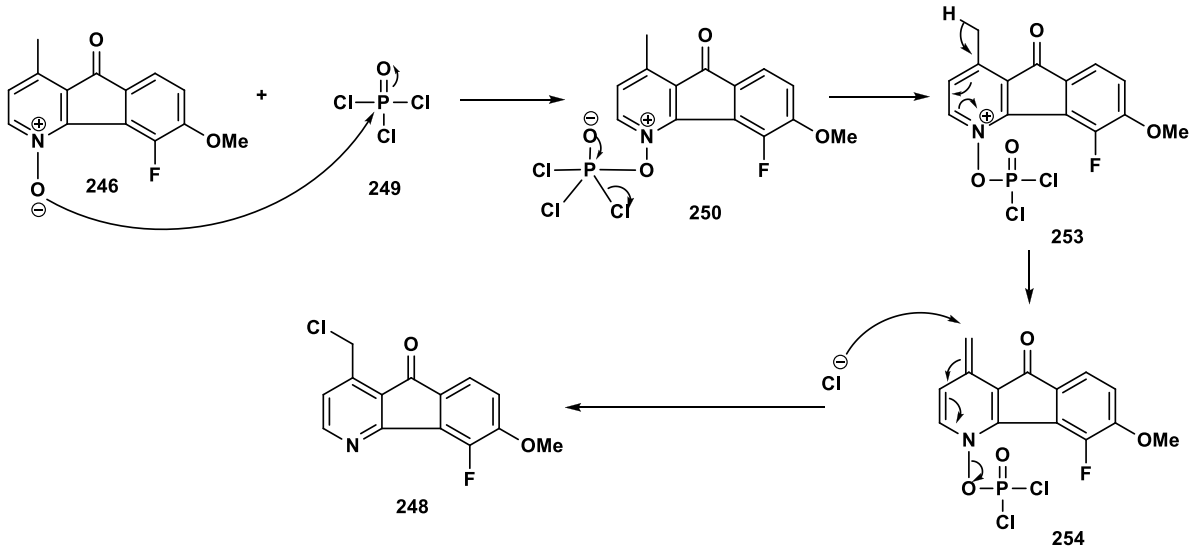


Figure 1.39 Proposed mechanism for the formation of **248**.

The formation of β-chlorinated product **247** may undergo the pathway shown in Figure 1.40. The β-carbon of **254** is also rendered electrophilic due to the presence of the good leaving group dichlorophosphate. Hence, the nucleophilic attack of chloride anion can also occur at the β-carbon to afford adduct **255**. Subsequent intramolecular proton transfer restores the aromaticity and furnishes chlorinated product **247**.

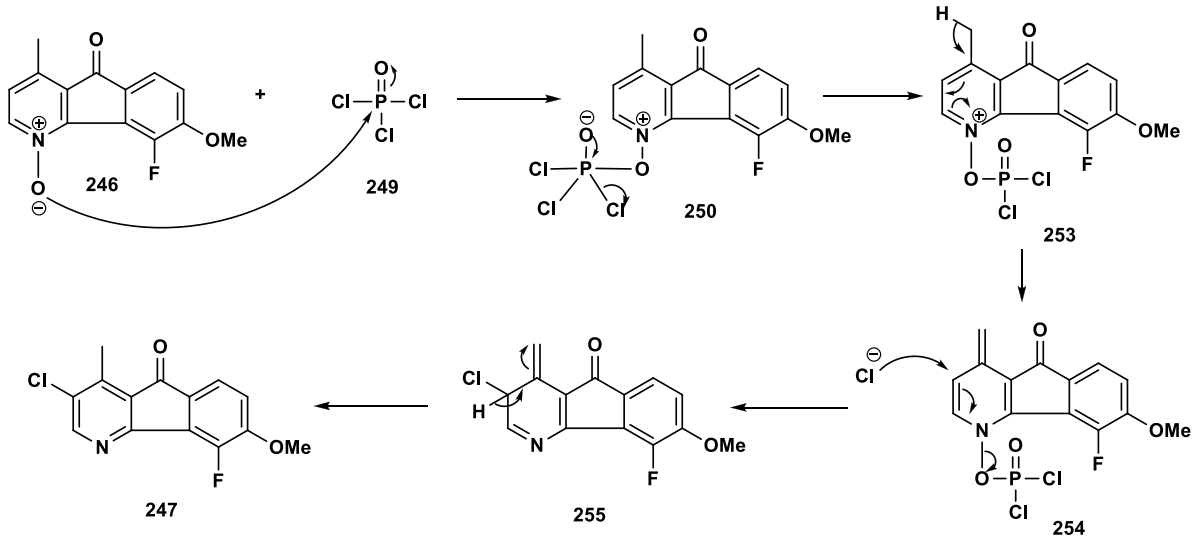
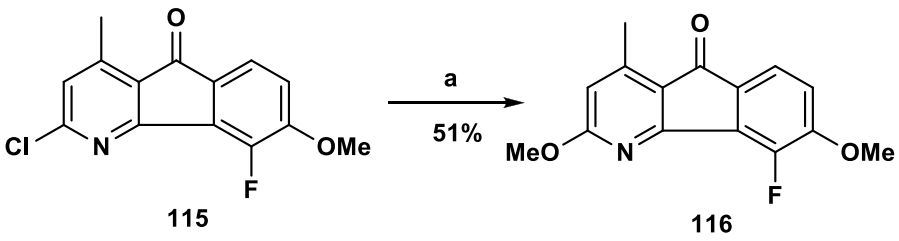


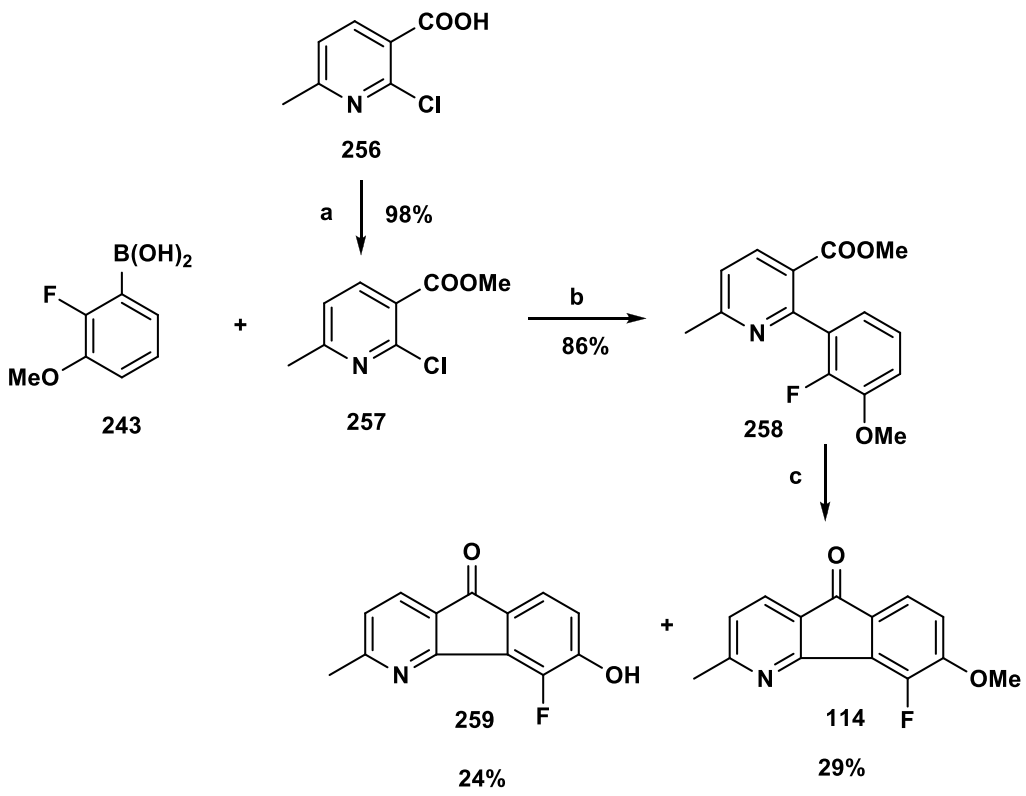
Figure 1.40 Proposed mechanism for the formation of **247**.

Methoxylation was achieved by the nucleophilic aromatic substitution *via* an addition-elimination mechanism. The treatment of chlorinated azafluorenone **115** with sodium methoxide in anhydrous methanol provided azafluorenone **116** in 51% yield (Scheme 1.45).



Scheme 1.45 *Reagents and conditions*: (a) NaOMe, MeOH, reflux.

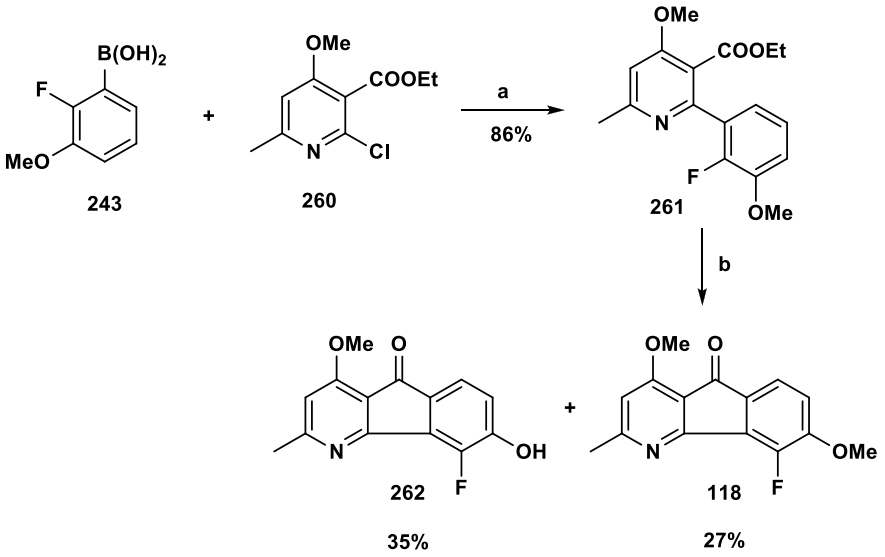
Starting from the 2-chloro-6-methylnicotinic acid **256**, azafluorenone **114** was prepared in a concise manner (Scheme 1.46). In the presence of potassium carbonate, nicotinic acid **256** was treated with iodomethane, affording ester **257** in excellent 98% yield. The formation of azabiaryl **258** was fulfilled by the palladium-catalysed cross-coupling of **257** and arylboronic acid **243** in the presence of potassium carbonate.[85] Subsequent PPA-promoted intramolecular acylation of **258** provided azafluorenones **114** and **259** in 29% and 24% yield, respectively.



Scheme 1.46 *Reagents and conditions*: (a) K_2CO_3, DMF, rt; (b) $Pd(PPh_3)_4$, THF, 2 M K_2CO_3 aq, 80 °C; (c) PPA, 130 °C.

The Suzuki cross-coupling of 2-chloro-4-methoxy-6-methylnicotinate **260** with arylboronic acid **243** afforded cyclisation precursor **261** in a yield of 86% (Scheme 1.47). Subsequent intramolecular cyclisation was accomplished through the treatment of **261** with polyphosphoric acid at 130 °C to afford azafluorenones **118** and

262 in 27% and 35% yield, respectively.



Scheme 1.47 *Reagents and conditions*: (a) $Pd(PPh_3)_4$, THF, 2 M K_2CO_3 aq, 80 °C; (b) PPA, 130 °C.

1.2.8 Synthesis of Azafluorenone Hybrid Molecules

The hybrid molecules will be prepared following the retrosynthetic analysis depicted in Figure 1.41. Starting from the commercial available nicotinate **265**, regioselective cross-coupling provides cyclisation precursor **264**. The azafluorenone framework is assembled by a ring closure of azabiaryl **264** to furnish the fused tricyclic ring system. The introduction of different amino side chains to the azafluorenone *via* a nucleophilic aromatic substitution completes the synthesis of hybrid molecules.

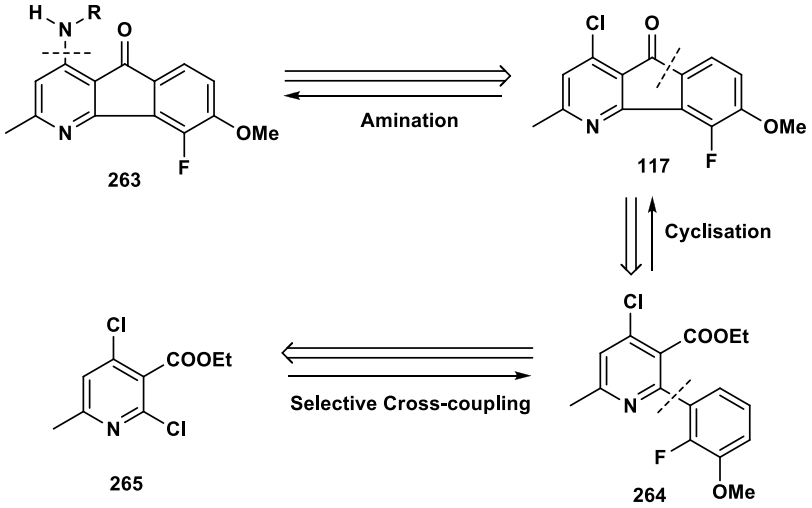
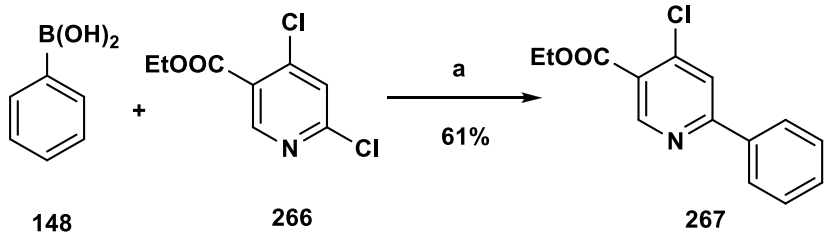


Figure 1.41 Retrosynthetic analysis towards hybrid molecules.

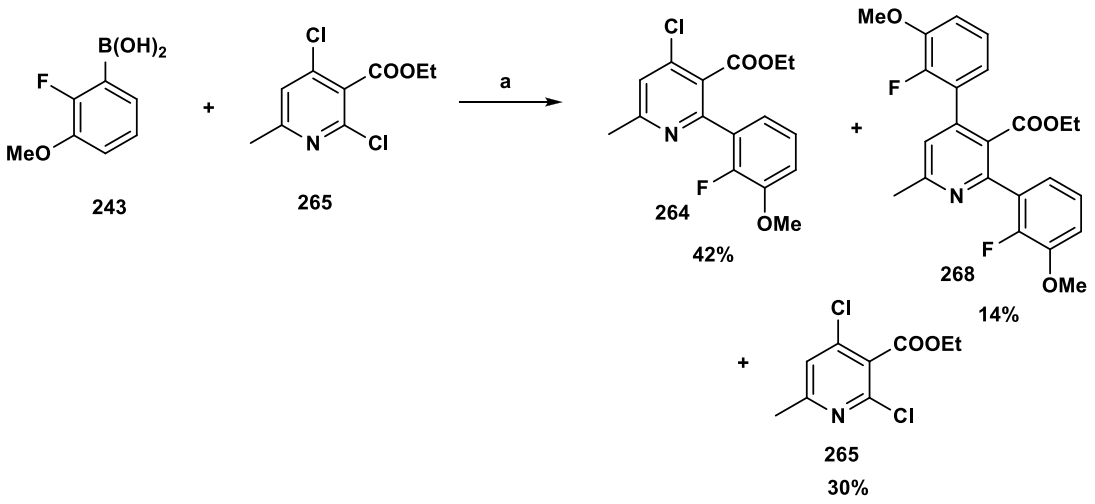
1.2.8.1 Regioselective Cross-coupling Reaction of Nicotinate 265

The key problem to be overcome in the proposed synthetic route is the regioselective cross-coupling of ethyl 2,4-dichloro-6-methylnicotinate **265**. Kohlmann has described a method for the selective Suzuki cross-coupling between nicotinate **266** and phenylboronic acid.[86] Catalysed by palladium reagent and potassium carbonate in a mixture of dimethoxyethane and isopropyl alcohol, cross-coupling is accomplished in 1 hour at 95 ºC, providing exclusively arylpyridine **267** in a yield of 61% (Scheme 1.48).



Scheme 1.48 *Reagents and conditions*: (a) $Pd(PPh_3)_4$, 2 M K_2CO_3 aq, DME, *i*PrOH, 95 °C, 1 h.

Employing the identical conditions, the Suzuki cross-coupling of nicotinate **265** and arylboronic acid **243** was performed. However, in addition to the desired mono-coupled product **264** being formed in 42% yield, the bis-coupled product **268** was also obtained in a yield of 14%, with recovery of significant starting material (30% yield) (Scheme 1.49).



Scheme 1.49 *Reagents and conditions*: (a) $Pd(PPh_3)_4$, 2 M K_2CO_3 aq, DME, *i*PrOH, 95 °C, 1 h.

^{13}C NMR spectroscopy has been utilised to confirm the structural identity of the mono-coupling product as azabiaryl **264**, rather than the 4-coupled isomer. In the ^{13}C NMR spectrum of nicotinate substrate **265**, the chemical shifts of C-2 and C-4 are 147.6 and 142.9 ppm, respectively (Figure 1.42). In the ^{13}C NMR spectrum of bis-coupled product **268**, the peaks for C-2 and C-4 are shifted to 152.8 and 143.8 ppm, respectively, suggesting the introduction of the aryl moiety brings about a downfield shift of signal relative to the original chlorinated carbon. In the ^{13}C NMR spectrum of mono-coupled product, the peaks for C-2 and C-4 are located at 153.2 and 142.1 ppm, respectively. The downfield shift of C-2 confirms that the mono-coupling reaction occurs at C-2, not C-4.

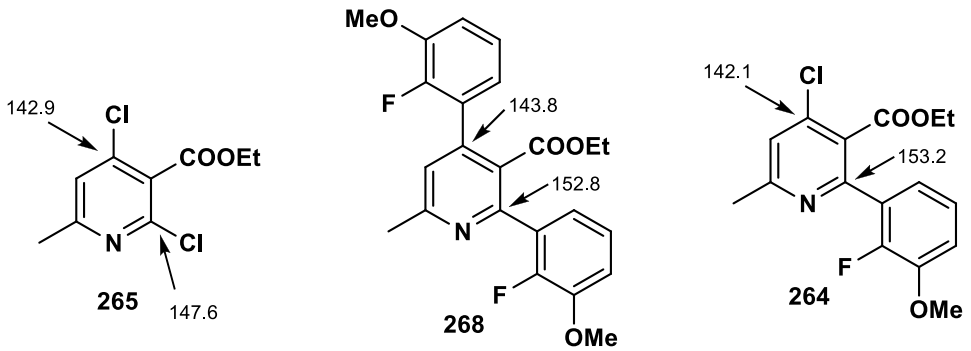
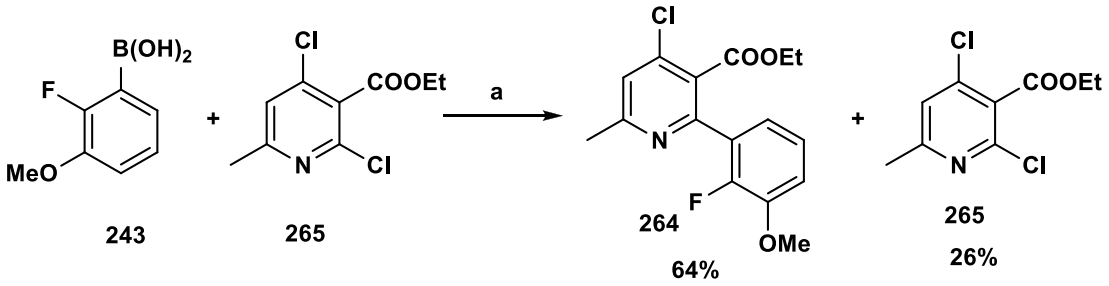


Figure 1.42 Chemical shifts of C-2 and C-4 in **264**, **265** and **268**.

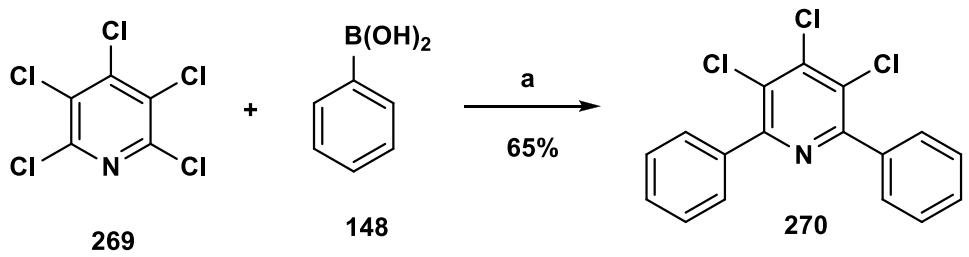
Different from the previous Suzuki cross-coupling reactions performed at 80 ºC, this cross-coupling was conducted at 95 ºC. It should be the higher energy provided by this increased temperature that facilitated the occurrence of the second cross-coupling at the less reactive 4-position in **264**.

It was decided to reduce the reaction temperature to avoid the undesired cross-coupling at the 4-position. Blackaby described a regioselective Suzuki cross-coupling between 2,4-dichloropyridine and 2-cyanophenylboronic acid at 80 ºC.[87] Accordingly, another attempt to preparation of azabiaryl **264** was made under these reaction conditions.[87] Although selective coupling occurred, there was still a significant amount of unreacted **265** recovered after 16 hours (Scheme 1.50).



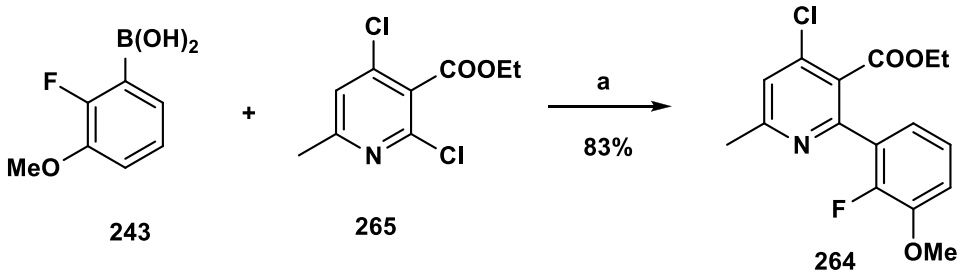
Scheme 1.50 *Reagents and conditions*: (a) $Pd(PPh_3)_4$, K_2CO_3, DME, CH_3CN, H_2O, 80 °C, 16 h.

Caesium carbonate, another widely utilised base in Suzuki cross-coupling reactions, has been shown to be efficient in regioselective cross-couplings of polychloropyridine (Scheme 1.51).[88] Therefore, caesium carbonate was also investigated for the selective preparation of azabiaryl **264**.



Scheme 1.51 *Reagents and conditions*: (a) $Pd(PPh_3)_4$, Cs_2CO_3, CH_3CN, H_2O, 100 °C.

In the presence of tetrakis(triphenylphosphine)palladium(0) and ceasium carbonate, treatment of nicotinate **265** with arylboronic acid **243** at 80 °C achieved the desired selective cross-coupling after 16 hours in a good yield of 83%, with no starting material **265** isolated (Scheme 1.52).



Scheme 1.52 *Reagents and conditions*: (a) $Pd(PPh_3)_4$, Cs_2CO_3, DME, CH_3CN, H_2O, 80 °C, 16 h.

These investigations confirm that the 2-position of nicotinate **265** is more reactive than the 4-position in Suzuki cross-coupling reactions. The regioselectivity should arise from the directing effect of the pyridine nitrogen (Figure 1.43). The lone pair of the ring nitrogen not participating in the resonance is available for pre-coordinating the palladium(0) reagent. This pre-coordination favours subsequent oxidative addition at the 2-position.[89] In addition, this coordination not only enhances the electron density of the palladium(0) reagent and its nucleophilicity, but also diminishes the electron density of the nitrogen and thus renders the 2-carbon more electron-deficient and more electrophilic, due to the stronger inductive effect of nitrogen. Nevertheless, there is no such directing effect of nitrogen in the case of oxidative addition at the 4-position. Consequently, the regioselectivity of cross-coupling is achieved finally.

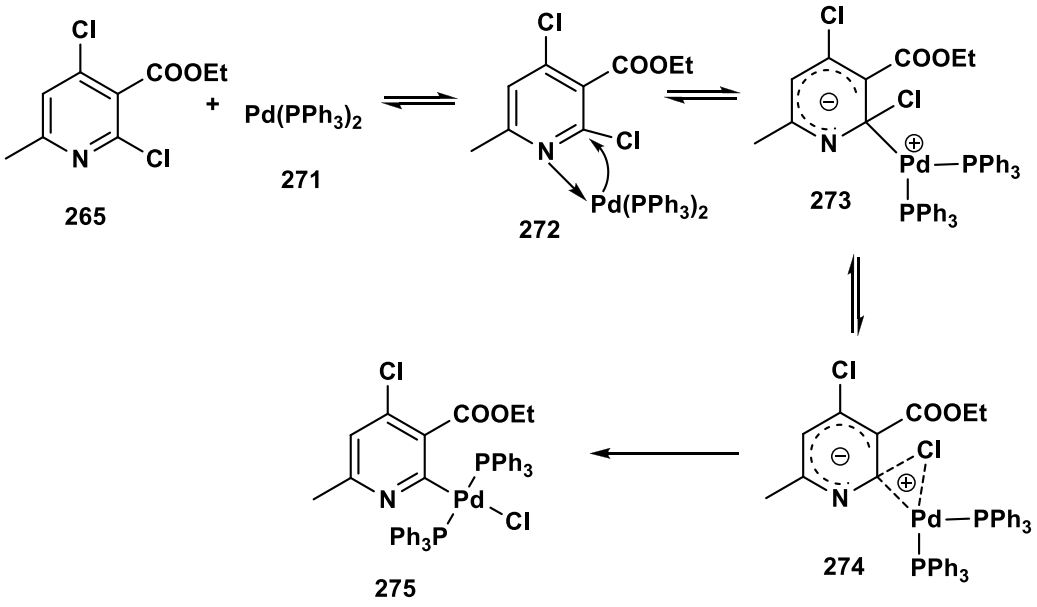


Figure 1.43 Directing effect of the pyridine nitrogen on the oxidative addition.

All the other conditions except the base employed are identical in the two Suzuki cross-couplings shown in Scheme 1.50 and Scheme 1.52. The different yields of these reactions imply that caesium carbonate exerts a more powerful effect upon the Suzuki cross-coupling than potassium carbonate.

To date, the selection of different bases for Suzuki cross-couplings is largely empirical. The origin of different effects by various bases remains to be elucidated and clarified.[90] In our opinion, the different effects observed for caesium carbonate and potassium carbonate may arise from different cation sizes, which ultimately results in the different quantities of hydroxide ion participating in the catalytic cycle. As displayed in Table 1.7, caesium cation possesses a much larger cationic radius than potassium cation.[91]

Cation	Li^+	Na^+	K^+	Rb^+	Cs^+
Cationic Radius (Å)	7.30	32.55	36.64	38.25	47.24

Table 1.7 Cationic radii of alkali metal ions.[91]

Investigations have shown two general principles with respect to ion pairing.[92] Firstly, association between cation and anion is stronger as the size of ions decrease. Secondly, there is a simple gradation in the association behaviour within the same metal family.

During the transmetallation step in the cross-coupling process, the quaternised arylboronic acid reacts with σ-aryl-Pd(II)-OH species **276**, accomplishing the nucleophilic transfer of the aryl group to the electrophilic palladium complex with the substitution of chloride. Hydroxide ions play a vital role in this process, by means of coordinating the arylboronic acid and enhancing the electron density as well as nucleophilicity of the boronic acid. Thus, the hydroxide ion derived from the hydrolysis of carbonate promotes the transmetallation between σ-aryl-Pd(II)-OH species **276** and arylboronic acid **243** (Figure 1.44).

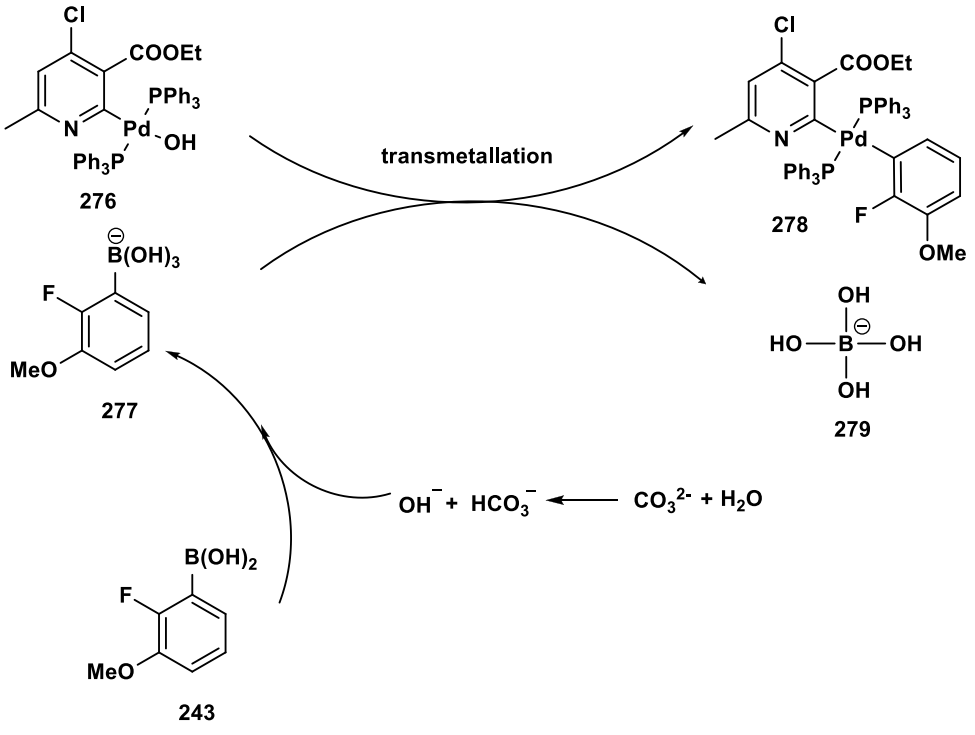


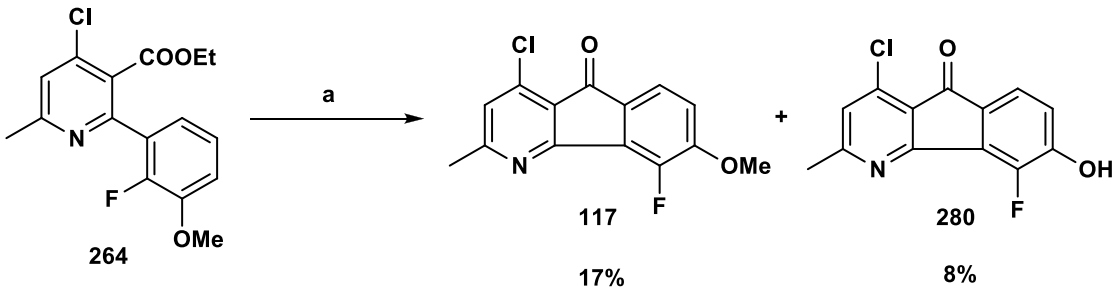
Figure 1.44 Function of the base in the transmetallation process.

Owing to the larger cationic radius of the caesium cation, caesium carbonate tends to dissociate to a greater extent than potassium carbonate, according to the foregoing ion

pairing principles.[92] It is the free carbonate ion that serves as the reactive species in the hydrolysis rather than the associated form.[93] Therefore, more hydroxide ions are yielded for the transmetallation in the case of caesium carbonate, leading to a more pronounced promoting effect on the Suzuki cross-coupling, in comparison to potassium carbonate.

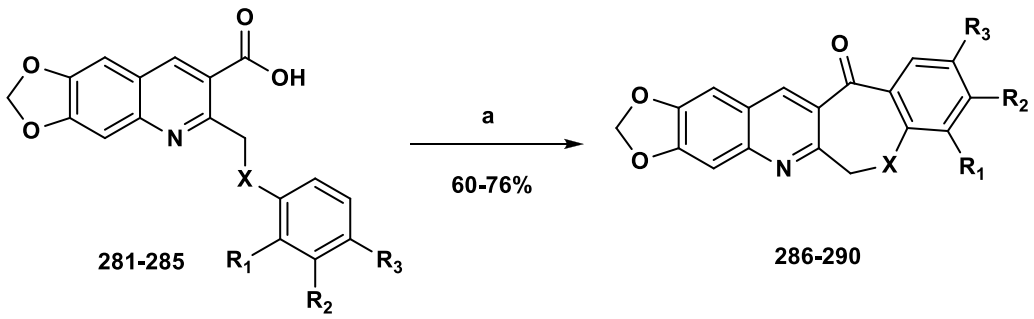
1.2.8.2 Cyclisation of Azabiaryl 264

Having obtained the target cross-coupling product **264**, the intramolecular acylation was conducted under our previously developed PPA-catalysed condition. However, the desired cyclisation product **117** and the demethylation product **280** were obtained in very poor yields (Scheme 1.53). This might be attributed to the instability of cyclisation precursor **264** or the instability of products **117** and **280** at the high reaction temperature.



Scheme 1.53 *Reagents and conditions*: (a) PPA, 130 °C.

Eaton's reagent, a combination of phosphorus pentoxide and methanesulfonic acid, acts as an efficient alternative for polyphosphoric acid in promoting intramolecular Friedel-Crafts acylations.[94] Li has described the utilisation of Eaton's reagent in the synthesis of fused quinolines (Scheme 1.54), fulfilling the intramolecular Friedel-Crafts acylations in mild to good yields (Table 1.8).[95]

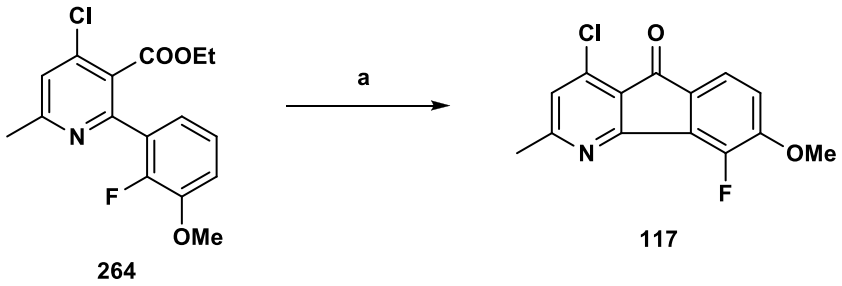


Scheme 1.54 *Reagents and conditions*: (a) Eaton's reagent, 80 °C.

Product	X	R_1	R_2	R_3	Yield (%)
286	O	Me	H	H	69
287	S	H	H	H	76
288	S	H	H	Me	70
289	O	H	OMe	H	63
290	O	H	H	Cl	60

Table 1.8 Eaton's reagent-mediated synthesis of fused quinolines.[95]

Accordingly, Eaton's reagent was employed for the cyclisation of azabiaryl **264** (Scheme 1.55). The intramolecular Friedel-Crafts acylation was optimised by varying the reaction temperature and time (Table 1.9). It has been proved that treatment of azabiaryl **264** with Eaton's reagent at 110 °C for 30 hours is the optimal conditions.

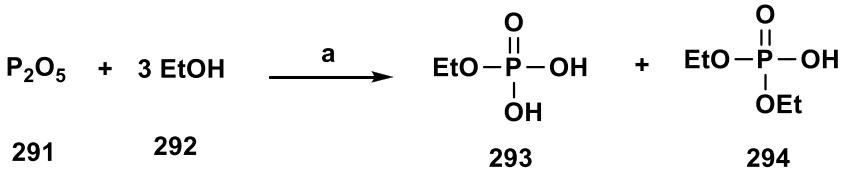


Scheme 1.55 *Reagents and conditions*: (a) Eaton's reagent, T °C.

Entry	T (°C)	t (h)	Yield (%)	Recovered Starting Material (%)
1	85	24	35	30
2	95	24	47	21
3	110	30	58	0

Table 1.9 Optimisation of Eaton's reagent-catalysed cyclisation.

It is noteworthy that the application of Eaton's reagent not only accomplished the cyclisation more efficiently than PPA did, but also avoided the demethylation of the azafluorenone **117**, which can be ascribed to the presence of phosphorus pentoxide in Eaton's reagent. Unlike polyphosphoric acid, there are no traces of water present in Eaton's reagent, due to the excellent desiccant capability of phosphorus pentoxide. In addition, phosphorus pentoxide is able to readily react with alcohols to yield mono- and di-alkyl phosphoric acid esters (Scheme 1.56).[96,97] Hence, the ethanol yielded during the cyclisation is consumed by phosphorus pentoxide, which facilitates the equilibrium of the intramolecular acylation shifting towards the azafluorenone formation, according to Le Chatelier's Principle.

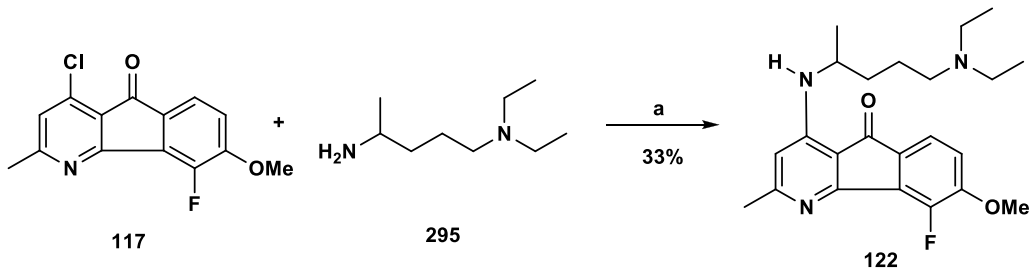


Scheme 1.56 *Reagents and conditions*: (a) 50 °C.

Consequently, the potential demethylation of the 6-methoxyl group in azafluorenone **117** induced by the generated ethanol or by the traces of water is completely precluded.

1.2.8.3 Amination of azafluorenone 117

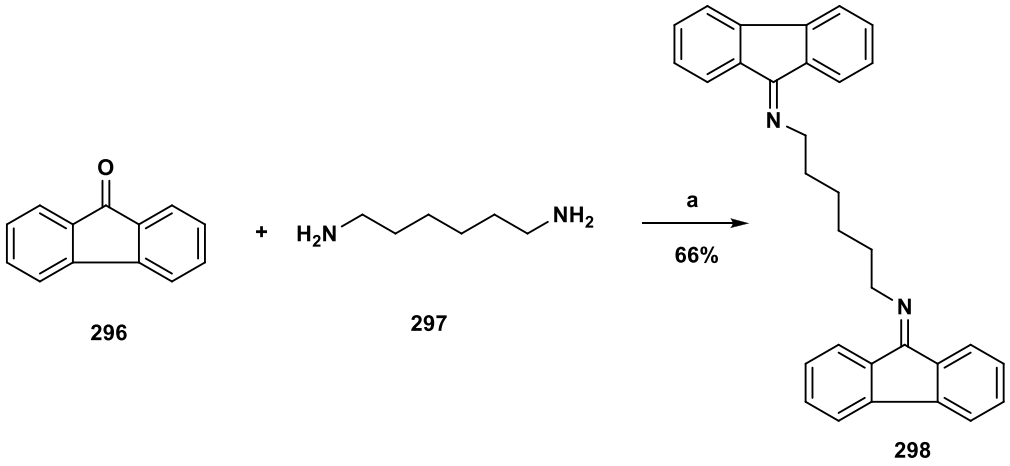
The introduction of a range of amino side chains was achieved by employing a similar method utilised in the synthesis of chloroquine.[98] Treatment of azafluorenone **117** with 2-amino-5-diethylaminopentane at 160 ºC in the presence of catalytic sodium iodide afforded the hybrid azafluorenone **122** in 33% yield (Scheme 1.57).



Scheme 1.57 *Reagents and conditions*: (a) NaI, PhOH, 160 °C.

When the crude product was purified by silica gel chromatography, a severe tailing was observed, resulting in a poor isolated yield (13%) of azafluorenone **122**. It is due to the fact the basic nitrogen atoms of the side chain strongly adsorb to acidic silica gel. Accordingly, neutral aluminium oxide was utilised for subsequent purification, providing the desired hybrid molecule **122** in an improved yield of 33%.

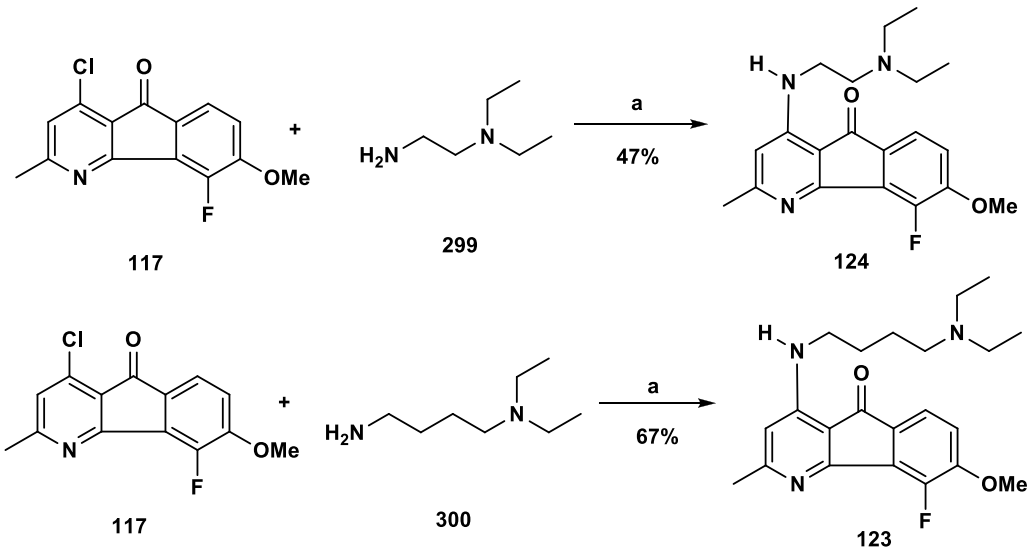
It is noteworthy that the carbonyl group in **117** provides an alternative reactive site leading to the formation of side products. It has been shown that even the relatively stable carbonyl group present in fluorenone **296**, is capable of reacting with primary amines to yield imines under Bronsted acid catalysis (Scheme 1.58).[99]



Scheme 1.58 *Reagents and conditions*: (a) AcOH, EtOH, 80 °C.

Although we were unable to isolate the corresponding imine derived from azafluorenone **117** due to the complex mixture of products formed, there is a good reason to speculate that the nucleophilic addition from amine **295** to the carbonyl group in **117** could be a competing side reaction giving the low yield of azafluorenone **122** observed.

Given the relatively low boiling point of 1-amino-2-diethylaminoethane (145-147 ºC),[100] the amination of **117** with the amino chain **299** had to be carried out at a reduced temperature. In the presence of catalytic sodium iodide, azafluorenone **117** reacted with 1-amino-2-diethylaminoethane in phenol at 130 ºC, giving hybrid product **124** in a yield of 47% (Scheme 1.59). Similarly, aminated azafluorenone **123** was obtained in 67% yield under the identical reaction conditions (Scheme 1.59).

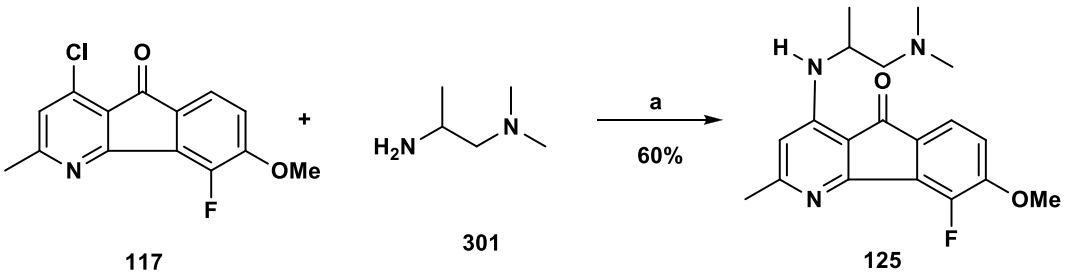


Scheme 1.59 *Reagents and conditions*: (a) NaI, PhOH, 130 °C.

The two aminations performed at the lower reaction temperature (130 °C) afforded the corresponding products in higher yields than the reaction involving amine **295** at 160 °C. Firstly, the absence of 1'-methyl group on these two amino chains may reduce steric hinderance during the nucleophilic aromatic substitution, which facilitates the amination. In addition, the lower reaction temperature may be unfavourable for the competing imination of the carbonyl group in **117**. Both of these two factors may contribute to the enhanced yield for the preparation of **123** and **124**.

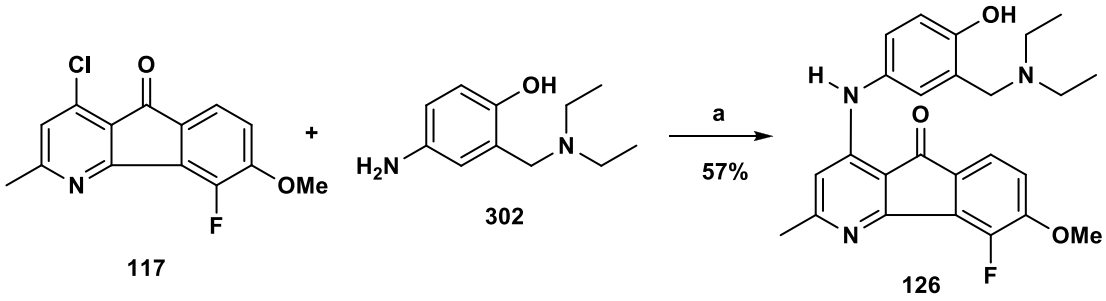
Since 2-amino-1-dimethylaminopropane possesses a even lower boiling point (110-111 ºC),[101] the reaction temperature was further reduced. Azafluorenone **117** was treated with amine **301**, under the catalysis of sodium iodide at 100 ºC, providing aminated product **125** in a yield of 60% (Scheme 1.60). The yield of azafluorenone **125** is higher than **124**, implying the reaction temperature is indeed a key factor influencing the competing imination. From the perspective of steric hinderance, the amination by **299** should have been favoured over the amination by **301**. However, the competing imination by **301** is retarded in comparison with **299**, due to the lower reaction temperature. The retardation of the competing imination may serve as the predominate

factor determining the yield of **125**, resulting in a higher yield of azafluorenone **125** than **124**.



Scheme 1.60 *Reagents and conditions*: (a) NaI, PhOH, 100 °C.

Finally, an aromatic side chain was appended to the azafluorenone utilising the method used in the synthesis of amodiaquine.[102] Treatment of azafluorenone **117** with 4-amino-2-diethylaminomethylphenol **302** at 120 ºC afforded the desired hybrid product **126** in a yield of 57%.



Scheme 1.61 *Reagents and conditions*: (a) 2-ethoxyethanol, 120 °C.

The presence of two nucleophilic centres in **302** enables the nucleophilic aromatic substitution to provide two plausible products. However, only the target azafluorenone **126** was obtained, which is attributable to two factors. Firstly, owing to the higher electronegativity, the hydroxyl oxygen nucleus possesses a stronger capability to absorb the extranuclear electrons than the amino nitrogen. As a result, the lone pair of the hydroxyl group is rendered less reactive than that of amino group, so that the amino group displays a higher nucleophilicity. Secondly, the presence of the tertiary alkylamino group at the *ortho*-position results in a steric hindrance to the hydroxyl group, which also precludes the nucleophilic attack of the phenol hydroxyl group.

1.3 Antiplasmodial Evaluation of Azafluorenones Analogues

Fourteen azafluorenones have been evaluated for antiplasmodial activity utilising a [^{3}H]hypoxanthine-incorporation assay by Miss Katharina Schuh in Professor Katja Becker's group at Justus Liebig University Giessen in Germany.

The [^{3}H]hypoxanthine-incorporation assay is the standard test for screening potential antimalarial agents.[103] Malaria parasites exploit exogenous purines in the synthesis of their nucleic acids. It has been demonstrated that hypoxanthine is the major purine base absorbed and utilised by *Plasmodium falciparum* as the precursor for the synthesis of adenosine and guanosine nucleotides and nucleic acids.[40] Thus, the utilisation of labelled hypoxanthine results in the incorporation of tritium into the parasite DNA during biosynthesis. The radioactivity measured, which represents the quantity of [^{3}H]hypoxanthine incorporated, is proportional to the growth of parasites in culture,[103] enabling this assay to be applied to the evaluation and development of potential antimalarial agents.

Fourteen azafluorenones were evaluated for antimalarial activity against both chloroquine-sensitive *Plasmodium falciparum* strain (3D7) and chloroquine-resistant *Plasmodium falciparum* strain (K1). In this assay, parasites were incubated with the azafluorenones at a parasitemia of 0.125% and 1.25% hematocrit, and 0.5 μCi of [^{3}H]hypoxanthine was added for the growth of parasites. The IC_{50} values of azafluorenones are summarised in Table 1.10.

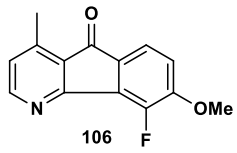
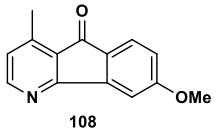
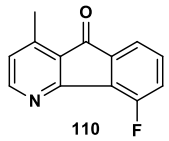
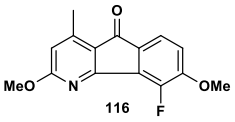
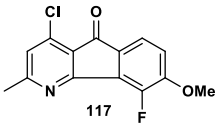
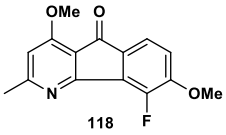
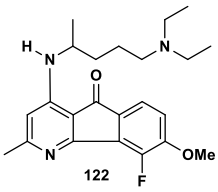
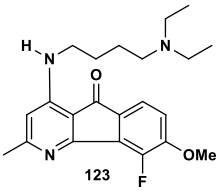
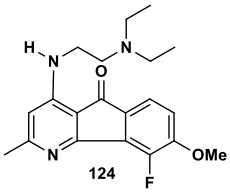
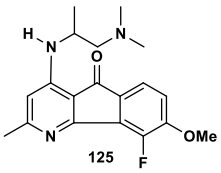
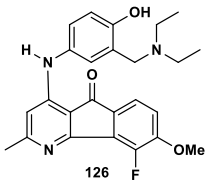
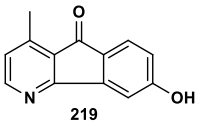
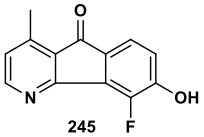
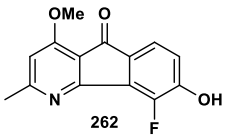
Compound	IC_{50} (3D7) (μM)	IC_{50} (K1) (μM)	Compound	IC_{50} (3D7) (μM)	IC_{50} (K1) (μM)
 106	18.6	16.2	 108	30.4 ± 0.48	> 44.0
 110	> 47.0	> 47.0	 116	> 37.0	> 37.0
 117	18.5	19.6	 118	> 37.0	22.9
 122	1.54	1.51	 123	2.16 ± 0.18	0.94
 124	2.42 ± 0.30	1.99	 125	2.45	7.32
 126	1.63	1.30	 219	> 47.0	> 47.0
 245	> 44.0	> 44.0	 262	> 39.0	33.3

Table 1.10 Antiplasmodial activity of azafluorenone analogues.

In general, the activity against the chloroquine-resistant strain K1 is similar to the activity against the chloroquine-sensitive strain 3D7 for each azafluorenone. The result

of the antiplasmodial activity assay shows a number of features allowing the elucidation of structure-activity relationship for azafluorenone analogues. Firstly, the 6-methoxy group is essential for antimalarial activity, as a loss of activity is observed when the 6-methoxy group is removed (azafluorenone **110** *versus* azafluorenone **106**) or displaced by hydroxyl group (azafluorenone **219** *versus* azafluorenone **108**, azafluorenone **245** *versus* azafluorenone **106**). Secondly, replacement of the 5-hydroxyl group in natural product **37** with the bioisostere fluorine retains the antiplasmodial activity (azafluorenone **106**). Thirdly, the substituent at the 5-position, which can serve as a hydrogen acceptor, is vital for the antimalarial activity, since the activity declines when the 5-position hydrogen acceptor is removed (azafluorenone **108** *versus* azafluorenone **106**). Fourthly, the presence of an electron-donating group present at the 3-position exerts a negative effect upon the activity, demonstrated by the comparison between azafluorenone **116** and azafluorenone **106**. The 3-substituent might interfere with the interaction between the azafluorenone and target site.

The effects of substituents at the 1-position are more complicated. The presence of hydrophobic groups at this site seem to be favourable towards the activity, as the analogue bearing the lipophilic chlorine (azafluorenone **117**) is more potent than the one with the comparatively hydrophilic methoxy group (azafluorenone **118**). However, the introduction of amino side chains to the 1-position induces a remarkable elevation of the antimalarial activity (azafluorenones **122-126**). There are two possible explanations for this result. If the mode of action of the hybrid molecules is inhibition of haemozoin formation within the digestive vacuole, the enhancement of the antiplasmodial activity could be attributed to the stronger interaction between these azafluorenones and haematins. In the acidic digestive vacuole, the protonated dialkyl tertiary amino group of the side chain in the hybrid molecule provides an additional electrostatic interaction with the carboxylate group of haematin, leading to the strengthened interplay between the azafluorenone and haematin and resulting in the improved antiplasmodial activity. If the hybrid molecules exert the antimalarial activity through binding to another target in the weakly basic cytoplasm of the parasite, the

uncharged amino groups might provide additional hydrogen bonding interactions with the target, promoting the binding of the azafluorenones to the target site and inducing the enhancement of the antiplasmodial activity.

The data shows that the variation of the amino side chains does not bring about a significant change of the activity. This can be ascribed to the fact that the aminoalkyl chains with different lengths are flexible. The flexibility allows the conformational adjustment of the side chains to adapt and match the binding position well, facilitating the effective interaction of azafluorenone and target. Therefore, the alternation of the side-chain length within a certain range will not result in a significant fluctuation of the antimalarial activity.

In order to acquire a comprehensive understanding of the effects exerted by diverse substituents at different positions, more azafluorenones will be prepared and evaluated. For example, the comparison among the antiplasmodial activities of azafluorenone **106**, azafluorenone **116** and 3-chloro-1-methyl-4-azafluoren-9-one **115** will further clarify the effects of functional groups at the 3-position.

1.4 Mechanism Exploration into the Action of Azafluorenones

To date, several modes of action have been identified for the current antimalarial agents, including inhibition of haemozoin polymerisation, alkylation of haem, and inhibition of dihydrofolate reductase or dihydropteroate synthase. Having synthesised a series of azafluorenone analogues, it is of great interest to explore the mechanism by which these molecules act utilising well-developed protocols.

1.4.1 Lipid Bodies-mediated Polymerisation of Haemozoin

During the intra-erythrocytic life stage, the parasite ingests haemoglobin of the host to acquire nutrients. In this catabolism process, considerable ferroprotoporphyrin IX (Fe(II)PPIX), known as haem, is also generated as a by-product (Figure 1.45). Ferroprotoporphyrin IX (Fe(II)PPIX) is converted to ferriprotoporphyrin IX (Fe(III)PPIX) readily in the digestive vacuole, which displays high toxicity towards the parasite by inducing lysis of malaria parasites.[104]

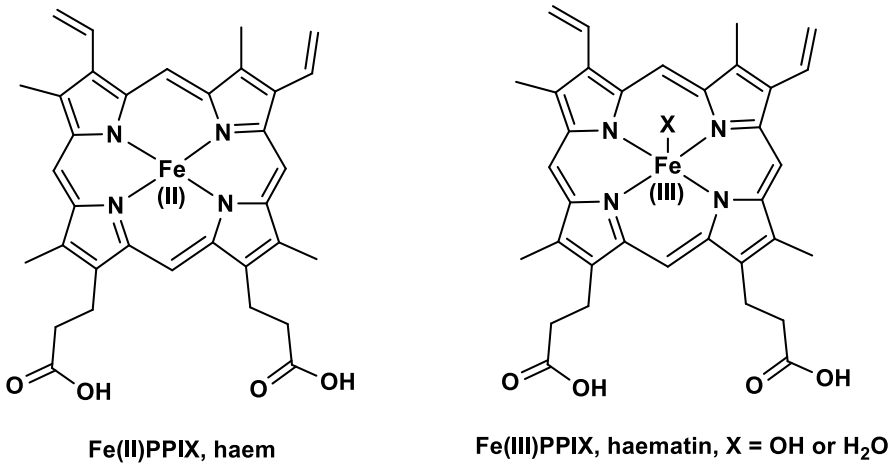


Figure 1.45 Structures of Fe(II)PPIX and Fe(III)PPIX.

In mammals, Fe(II)PPIX is catabolised to bilirubin in two steps (Figure 1.46).[105] Under the action of NADPH and haem oxygenase, the tetrapyrrolic ring of Fe(II)PPIX is initially cleaved to afford biliverdin **304**, with the liberation of carbon monoxide and ferrous ion. Biliverdin **304** is further converted to bilirubin **305** by biliverdin reductase,

in the presence of NADPH.

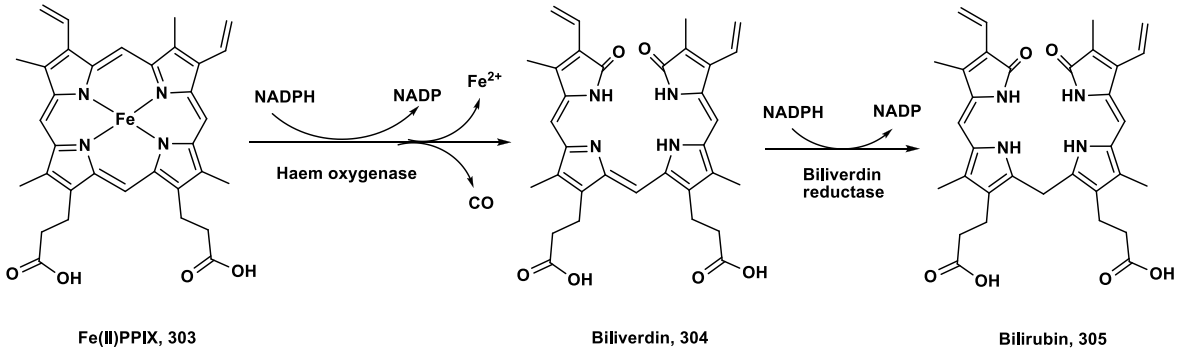


Figure 1.46 Catabolism of haem in mammals.

The malaria parasite does not possess the haem oxygenase enzyme and has to circumvent haem toxicity through the polymerisation of ferroprotoporphyrin IX molecules into the innocuous biomineral haemozoin. Haemozoin, also known as the malaria pigment, is a crystal composed of reciprocal head-to-tail dimeric units of ferriprotoporphyrin IX (Figure 1.47). The dimeric unit consists of two Fe(III)PPIX molecules connected by the linkages between the propionate oxygen atom in one monomer and the iron centre in the other monomer. Haemozoin is a polymer formed *via* the hydrogen bonds between different dimeric units.

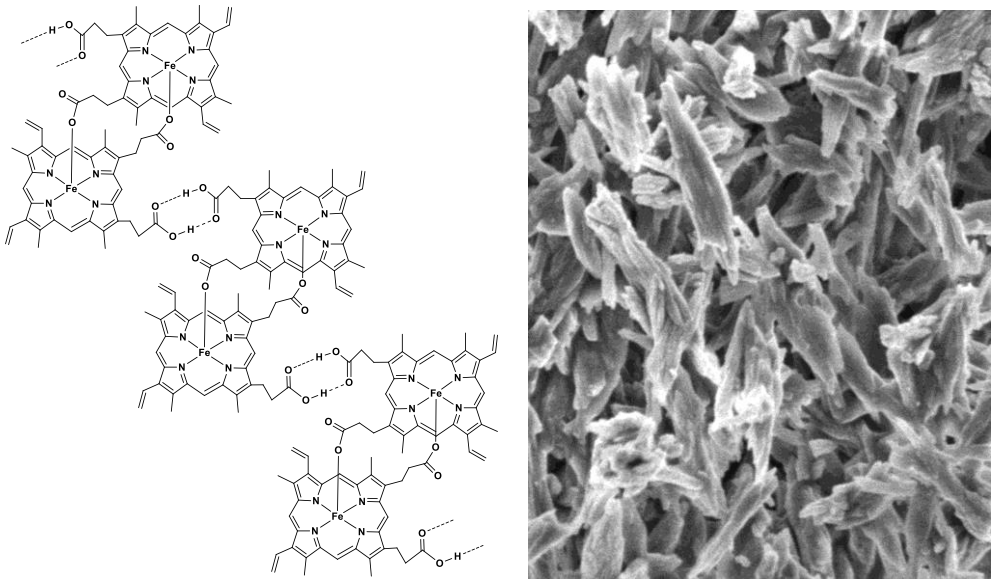


Figure 1.47 Structure and scanning electron microscopy of haemozoin.[106]

Egan has proposed the mechanism for the generation of haemozoin precursor, which is shown in Figure 1.48. Two molecules of H_2O-Fe(III)PPIX, derived from Fe(II)PPIX molecules, could form a π-stacked dimer **306**. The shift of two monomers forms dimer **307**, which facilitates the interaction between the propionate groups and iron centres. Subsequent ligand exchange and the displacement of the axial water molecules afford the head-to-tail dimer **309**, which acts as the unit cell of haemozoin.[106]

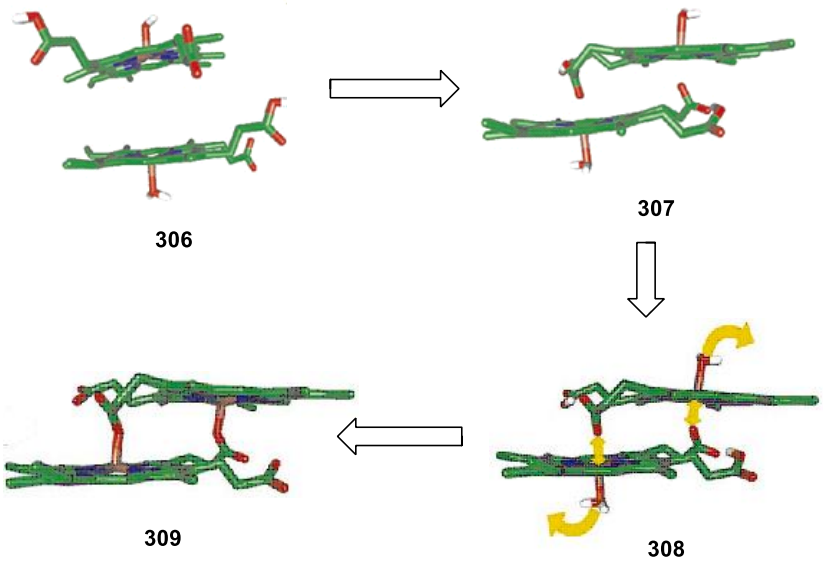


Figure 1.48 Proposed mechanism for the generation of dimeric unit of haemozoin.[106]

With the formation of the unit cell of haemozoin, further interactions between propionic acid groups from different dimeric units constitute an extended network of hydrogen bonds, achieving the assembly of the haemozoin crystal.[106] The complete course of the haemozoin formation is shown in Figure 1.49.

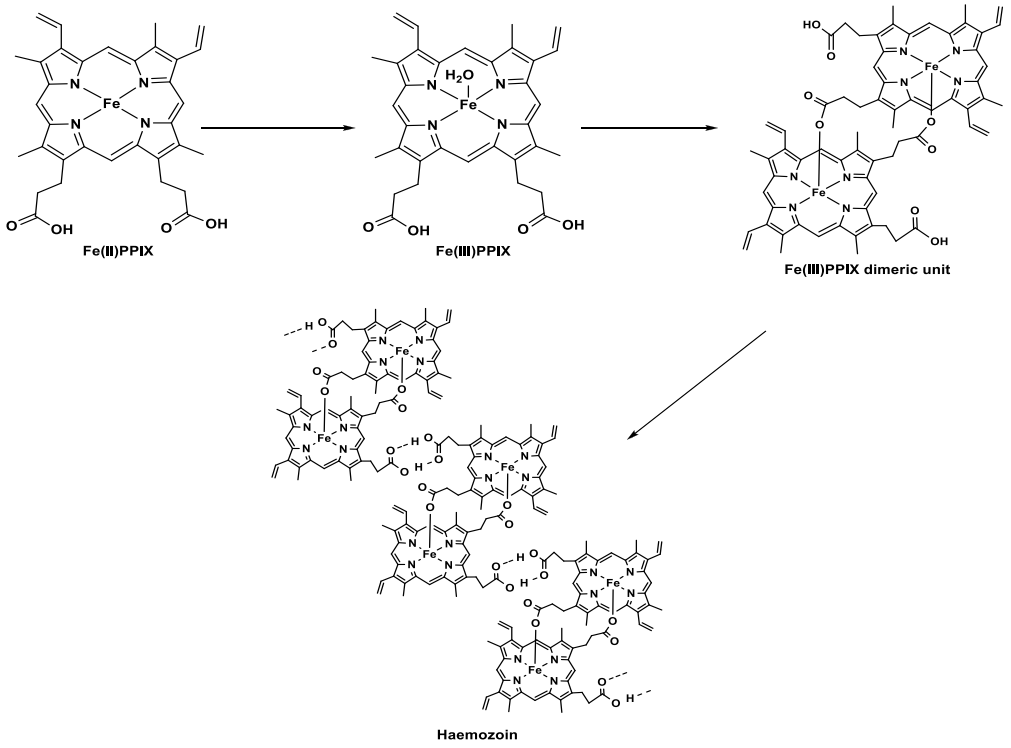


Figure 1.49 Pathway of haemozoin formation.

Recent studies have shown that neutral lipid bodies present in the parasite play a vital role in the biocrystallisation of haemozoin. Lipid bodies, also known as lipid particles, are intracellular storage compartments for neutral lipids.[107] Within the digestive vacuole of the parasite, a large quantity of monoglyceride and diglyceride is synthesised and packaged into neutral lipid bodies. This specialised organelle is believed to promote the process of haemozoin formation by serving as the scaffold and site for the nucleation and crystal growth of haemozoin.[108,109] Hoang believes that the mono- and diglyceride molecules stored in lipid bodies assemble with their polar hydroxyl groups facing outwards, forming the interface between lipid bodies and water. Fe(III)PPIX molecules accumulate at the surface of the lipid bodies. The hydroxyl groups exposed at the surface interact with Fe(III)PPIX molecules, allowing the pre-organisation of Fe(III)PPIX molecules to take place.[109] This pre-organisation facilitates the further interaction between Fe(III)PPIX molecules and promotes nucleation.[109]

1.4.2 *In Vitro* β-Haematin Inhibition Assay

Numerous investigations have shown that a range of antimalarial drugs, including chloroquine, amodiaquine and quinine exert the antiplasmodial activity *via* inhibition of haemozoin formation. By forming a drug-haem complex, which adsorbs onto the actively growing face of haemozoin, these antimalarial agents cap the haemozoin crystal and impede the extension of polymer.[6] As a consequence, toxic free haems accumulate within the *Plasmodium*, prompting the death of parasite. The resistance towards chloroquine has been proved to occur *via* a structure-specific efflux mechanism.[5,108] The mutated PfCRT is able to recognise chloroquine and facilitate the efflux of chloroquine from the digestive vacuole.[5,108] Since the occurrence of chloroquine resistance does not directly originate from the process of haemozoin inhibition,[5] inhibition of haemozoin biomineralisation still remains a significant and viable target for the development of antimalarial agents.[108]

As a synthetic polymer, β-haematin is structurally identical to the native haemozoin.[110] *In vitro* assays evaluating the inhibitory capabilities of compounds against β-haematin formation can serve as a feasible and efficient approach for the evaluation of antimalarial compounds. In Carter's study, various detergents have been examined for their capacity for mediating formation of β-haematin *in vitro*, among which detergent Nonidet P-40 (NP-40) has been shown to be the optimal promoter mimicking the native lipid bodies in the digestive vacuole.[110] The azafluorenone analogues synthesised share some structural features in common with the haemozoin inhibitor chloroquine, both possessed a conjugated planar core. In order to probe the mechanism of azafluorenone antimalarial activity, it was decided to explore their inhibitory activity against β-haematin formation utilising the NP-40 detergent-mediated assay.[110]

Prior to the evaluation of the inhibitory capacity of azafluorenones, a model assay employing the positive control amodiaquine was conducted to probe the reproducibility of the method. Nine different concentrations of amodiaquine similar to those tested in

Carter's trial were selected in our assay. Haematin was incubated under the established conditions to allow β-haematin formation to occur.[110] The different solubilities of haematin and β-haematin in the mixed solution of sodium bicarbonate and sodium dodecyl sulphate (SDS) served as the basis for this protocol and were exploited in the purification of β-haematin product after incubation. After the centrifugation and removal of the supernatant, the solution of sodium bicarbonate and sodium dodecyl sulphate was added to selectively dissolve the unconverted haematin monomer in the mixture of haematin and β-haematin. The further centrifugation achieved the separation between β-haematin precipitate and haematin. The purified β-haematin was finally dissolved in the solution of sodium hydroxide and sodium dodecyl sulphate, whose absorbance was measured at 400 nm. The quantity of β-haematin was calculated according to the reported extinction coefficient.[110]

However, significant defects were identified in this protocol. Firstly, the triplicate data for each concentration of amodiaquine deviated significantly, indicating poor reproducibility of this method. The triplicate raw data of absorbance for each concentration are displayed in Table 1.11. This deviation originates from the two steps of supernatant removal. It was found that the sedimentation of β-haematin after the centrifugations was poor, which made the supernatant removal difficult to achieve. β-Haematin precipitate floated up during pipetting and was discarded with the supernatant, giving rise to the inaccuracy of the subsequent quantification of β-haematin.

C(μM) / **Absorbance**	411.2	205.6	102.8	51.4	25.7	12.85	6.43
1	0.310	0.477	0.298	0.323	0.633	1.247	1.659
2	0.178	0.256	0.623	0.387	0.524	1.172	1.861
3	0.136	0.382	0.515	0.586	0.481	1.528	2.037

Table 1.11 Raw data of absorbance for assay of β-haematin inhibitory activity.

In addition, the complicated procedure involving twice lengthy centrifugations also reduces the efficiency of this protocol, rendering this method inappropriate for automated high-throughput screening.

To resolve these problems, another protocol quantifying the unconverted haematin, instead of β-haematin, has been developed for the evaluation of β-haematin inhibitory activity.[108] It has been demonstrated that in an aqueous solution containing 5-10% (v/v) pyridine, buffered at neutral or mildly basic pH, pyridine molecules are able to selectively coordinate the iron centre in the Fe(III)PPIX monomer molecule and form a pyridine-haematin complex.[105] This complex possesses a strong and distinctive absorption at 405 nm and exhibits a characteristic orange-pink colour.[111] However, pyridine solution at this concentration and pH cannot react with β-haematin. This special absorption obeys Beer's Law, allowing the quantitative determination of free haematin in a mixture of haematin and β-haematin.

Accordingly, assessment of β-haematin inhibitory activity of azafluorenones was modified according to the colorimetric pyridine protocol developed by Sandlin.[108] Model assays evaluating the efficacy of the positive controls amodiaquine and quinine in inhibiting β-haematin formation were performed initially. After the incubation of haematin with a range of concentrations of amodiaquine or quinine, the assay was analysed utilising the colorimetric pyridine-based method, with the addition of the previously prepared pyridine solution. The absorbance of the resulting complex was finally measured at 405 nm.

The inhibition percentage for this assay is calculated according to the following equation:

$$I = (A_s - A_{in})/(A_0 - A_{in}) \times 100\%$$

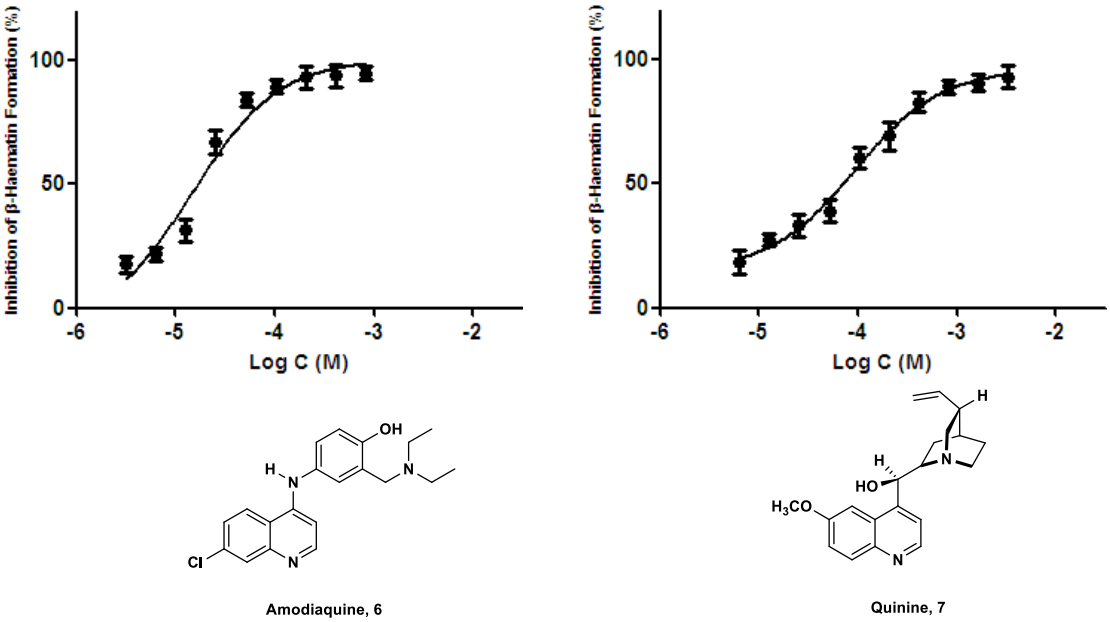
I: inhibition percentage

A_s: absorbance of the solution with haematin and tested drug incubated in the presence of NP-40;

A_{in}: absorbance of haematin incubated in the presence of NP-40;

A_0: absorbance of haematin in the absence of tested drug and NP-40.

Based upon the inhibition percentage calculated from the raw data of absorbance at 405 nm, sigmoidal concentration response curves as well as the IC_{50} values were acquired using GraphPad Prism v5.0 (Figure 1.51).



IC_{50} = 20.3 μM

IC_{50} = 83.9 μM

Figure 1.51 Inhibitory activities of AQ and QN against β-haematin formation.

As shown in Figure 1.51, both amodiaquine and quinine exhibit potent inhibitory activity against β-haematin formation. Amodiaquine possesses an IC_{50} value of 20.3 μM, quinine possesses an IC_{50} value of 83.9 μM, suggesting that amodiaquine has a higher inhibitory activity against β-haematin formation than quinine does. The IC_{50} value of amodiaquine acquired from this model assay correlates with the value obtained in the literature using the same method (21.0 μM).[108] This validates the reproducibility of the colorimetric pyridine method.

In this assay, sodium acetate-acetic acid buffer solution at pH 4.9 and the incubation at 37 °C furnish a physiological environment resembling that within the parasite's acidic digestive vacuole. Thus, crystallisation of β-haematin was promoted by detergent NP-40, which served as a mimic for neutral lipid bodies.

Nonidet P-40 is a nonionic surfactant possessing a hydrophilic polyether chain and a hydrophobic alkylphenyl group (Figure 1.52). It is the hydrophilic head group and the lipophilic tail group within NP-40 that enable these surfactant molecules to function similarly to the amphiphilic neutral lipid bodies present in the digestive vacuole. In the acetate buffer solution, NP-40 molecules organise into spherical aggregates with their polar and hydrophilic head groups facing outwards.[112] In this way, NP-40 molecules interact with Fe(III)PPIX molecules *via* the hydrogen bonding between the polar head groups of NP-40 and the propionic acid groups of Fe(III)PPIX, which results in the accumulation of Fe(III)PPIX molecules on the surface of spherical NP-40 aggregates. Once the dimeric unit of Fe(III)PPIX is formed, it can enter the spherical structure, where the lipophilic tail groups provide a hydrophobic environment stabilising and protecting this unit cell of β-haematin by precluding the attack from water molecules to the iron centre which gives rise to the dissociation of this dimer. These hydrophobic surroundings can also favour the construction of the hydrogen bonding network between different dimeric Fe(III)PPIX units *via* obviating the competitive hydrogen bonding with water molecules.

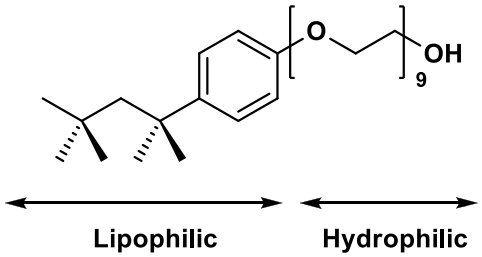


Figure 1.52 Structure of Nonidet P-40 molecule.

In comparison with the method described by Carter,[110] the new pyridine-based protocol avoids the difficult and time-consuming manual removal of the supernatant and does not require lengthy centrifugations, significantly enhancing the efficiency of this assay. More importantly, the convenient manipulation of the colorimetric pyridine-based method makes this an ideal approach for the high-throughput screening (HTS) of β-haematin inhibitors. Promising inhibitors identified using this assay can then be further profiled in antiplasmodial assays to evaluate their antimalarial potency.

Having verified the reliability and reproducibility of the colorimetric pyridine-based method, four different concentrations of each azafluorenone, ranging from 1.645 mM to 205.6 μM, were used to evaluate their inhibitory potency against β-haematin formation in a preliminary assay. The compounds showing promising activity were then investigated more thoroughly and further profiled at nine concentrations to provide accurate IC_{50} values. In the assays, amodiaquine was employed as the positive control drug. The results of these tests are shown in bar charts in Appendix I.

However, most of the azafluorenones tested displayed very poor inhibitory activity against β-haematin formation. Only azafluorenones **245** and **262** exhibited some inhibitory potency. On the basis of the inhibition percentage calculated from the data obtained in the further assays, sigmoidal concentration response curves as well as the IC_{50} values were acquired by utilising GraphPad Prism v5.0 (Figure 1.54).

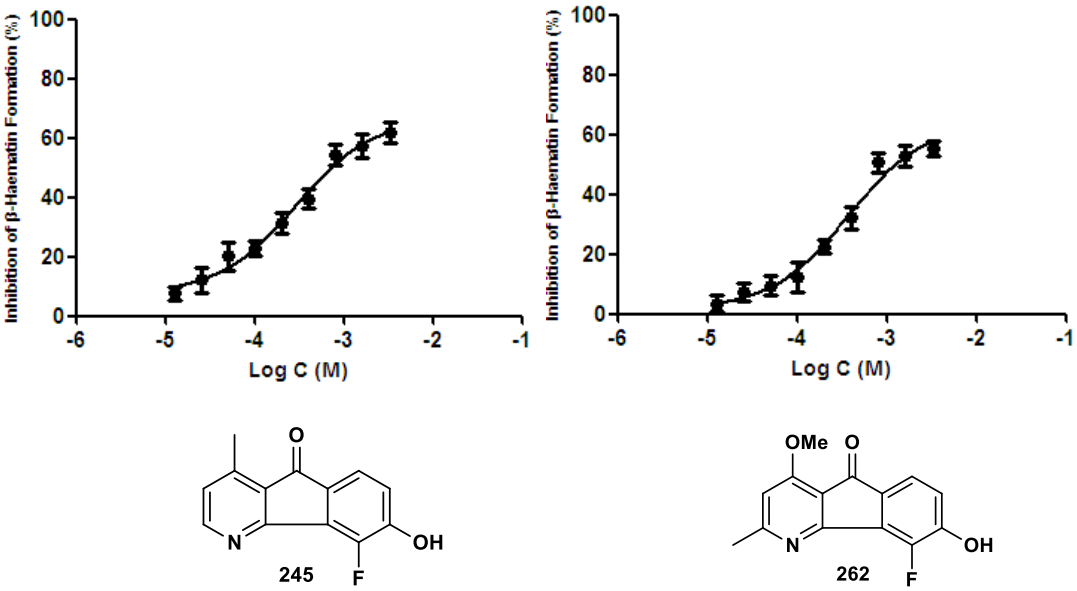


Figure 1.54 Inhibitory activity of **245** and **262** against β-haematin formation.

In comparison with the other azafluorenone anologues, **245** and **262** display much higher inhibitory activity against the β-haematin formation, with IC_{50} value at 521.2 and 685.8 μM, respectively. This enhancement in activity is attributable to the presence of the hydroxyl group in both molecules. The planar azafluorenone core can form a π-π stacking interaction with the porphyrin in Fe(III)PPIX molecule, while the 6-hydroxyl group provides an additional hydrogen bonding interaction with carboxyl acid group in Fe(III)PPIX, reinforcing the overall interaction between the azafluorenone and Fe(III)PPIX. Thus, the azafluorenone-Fe(III)PPIX complex formed can adsorb onto the growing face of β-haematin, capping the β-haematin crystal and precluding the extension of polymer. The slightly improved inhibitory activity which might originates from this hydrogen bonding interaction is also observed in the comparison between azafluorenones **259** and **114**, **219** and **108**.

However, the results of the *in vitro* β-haematin inhibition assays reveal no correlation with the data of the antiplasmodial assays. For example, azafluorenone **245** is the most active β-haematin inhibitors among the analogues synthesised, whereas it exhibits a

poor antiplasmodial activity in the tests conducted by Prof Becker's group. Azafluorenone **106** displays a higher antimalarial activity than azafluorenone **262**, while azafluorenone **262** is a stronger β-haematin inhibitor than azafluorenone **106**. These results along with the poor inhibitory activity of most of the analogues prepared imply that the azafluorenone class might act *via* another mechanism other than the inhibition of β-haematin formation.

A series of azabiaryls, the precursors of the azafluorenones, were also evaluated for the inhibitory activity against the formation of β-haematin by this assay. However, none of these compounds displayed significant activity. The results of these tests are displayed in bar charts in Appendix II. The single carbon-carbon bond connecting the two rings in the azabiaryl makes the intramolecular rotation of the two moieties possible to occur. Hence, the π-π stacking interaction between the azabiaryl and Fe(III)PPIX molecule is weaker due to the absence of the rigid planar tricyclic backbone. This could explain their poor inhibitory activity against β-haematin formation.

Since most of the azafluorenones displays poor inhibitory activity against β-haematin formation, it is necessary to consider other mechanisms which might be responsible for the antiplasmodial activity of azafluorenones.

1.5 Summary and Conclusions

To explore the structure-activity relationship of the azafluorenone family of alkaloids, twenty four analogues with various structural modifications have been synthesised. Palladium-catalysed Suzuki-Miyaura cross-coupling reactions were exploited to prepare the azabiaryl cyclisation precursors in good to excellent yields. Polyphosphoric acid catalysed Friedel-Crafts acylations were then applied to the intramolecular cyclisations to construct the conjugated tricyclic ring framework. Structural

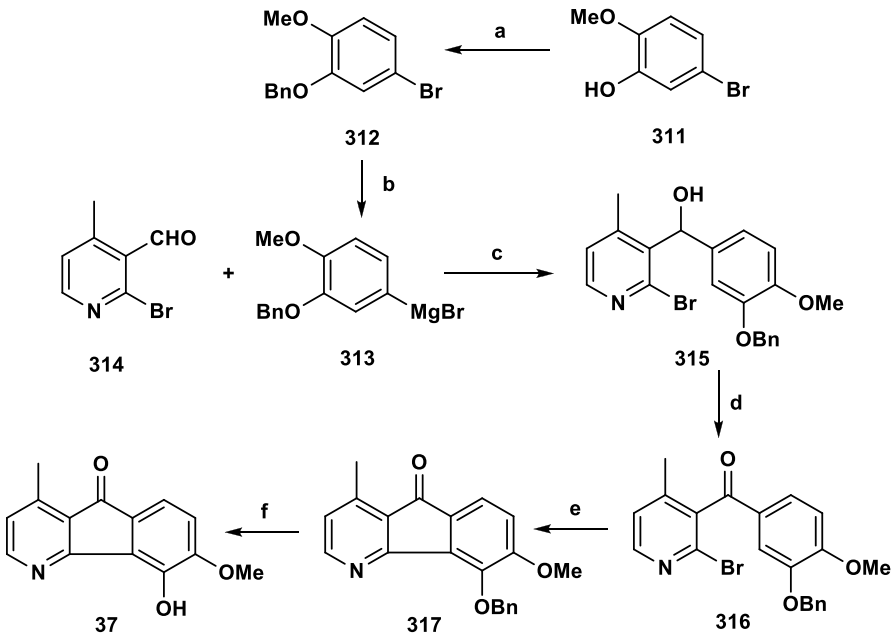
modifications at the 3-position were achieved *via* a linear synthetic route, involving oxidation, phosphorus oxychloride-mediated chlorination and nucleophilic aromatic substitution. A series of hybrid molecules conjugating the azafluorenone core and the amino side chains present in other antimalarial agents were also prepared. The synthesis of these hybrid molecules was fulfilled *via* the selective Suzuki-Miyaura cross-coupling, Eaton's reagent-promoted cyclisation and the subsequent nucleophilic aromatic substitution. Four distinct synthetic routes were investigated for the total synthesis of the parent antimalarial natural product **37**. However, each of these failed to afford the desired natural product.

Fourteen of the synthesised azafluorenone analogues were evaluated for antiplasmodial activity by Prof Katja Becker. The [^{3}H]hypoxanthine-incorporation assay revealed that eight azafluorenones exhibited activity against chloroquine-sensitive *Plasmodium falciparum* 3D7, and nine azafluorenones were active against chloroquine-resistant *Plasmodium falciparum* K1. The analysis of the antiplasmodial data provides several significant conclusions for the structure-activity relationship of the azafluorenones. Replacement of the 5-hydroxyl group in natural product **37** with bioisostere fluorine retains the antiplasmodial activity of azafluorenones. The substituent at the 5-position, which can serve as a hydrogen acceptor, and the 6-methoxy group of the azafluorenone analogues are essential for the antimalarial activity. In addition, the hybrid molecules prepared were proved to possess a much higher potency than the other azafluorenones. The amino side chains appended might bring about additional interaction with the target site, significantly facilitating the binding of the azafluorenones to the target.

To probe the mode of action of the azafluorenones, an *in vitro* β-haematin inhibition assay was conducted. It was found that only two azafluorenones showed mild inhibitory activity. Moreover, no correlation was identified between the result of antiplasmodial activity and that of the inhibition of β-haematin formation, suggesting these analogues might act *via* another mechanism other than the inhibition of haemozoin formation.

1.6 Plans for Future Work

Our attempts to prepare natural product **37** suggest that the synthetic route involving the cross-coupling and PPA-catalysed intramolecular Friedel-Crafts acylation may be inappropriate for the synthesis of alkaloid **37**. Another synthetic route with Grignard reaction and intramolecular Heck reaction as the key steps can be applied to the preparation of natural product **37** (Scheme 1.62).[52] The protection of the free hydroxyl group with a benzyl group will provide a base-tolerant substrate for the preparation of Grignard reagent **313**. Subsequent Grignard reaction will conjugate the pyridyl and phenyl moieties at the β-position. The oxidation of **315** will yield the precursor for the intramolecular cyclisation. Subsequent Heck reaction will ultimately achieve the construction of the tricyclic ring system. Removal of the benzyl group will provide the target alkaloid **37**.



Scheme 1.62 *Reagents and conditions*: (a) BnBr, K_2CO_3, acetone; (b) Mg, THF, reflux; (c) ether, 0 °C; (d) MnO_2, toluene, 60 °C; (e) $Pd(OAc)_2$, NaOAc, DMF, 100 °C; (f) 10% Pd/C, H_2, 1 atm, MeOH, rt.

An important issue that cannot be neglected for hybrid molecule **126** is the potential hepatotoxicity originating from the aromatic side chain of amodiaquine.[9] The *p*-aminophenol moiety present in amodiaquine is prone to undergo the biotransformation to the quinone imine **318** (Figure 1.58), which is an active electrophilic metabolite showing a high affinity for the thiol groups in proteins. The nucleophilic attack of a thiol group to quinone imine **318** results in the conjugation of amodiaquine to the protein, giving rise to hepatotoxicity.[5,9] Hence, it is necessary to make modifications of the *p*-aminophenol moiety in **126** to avoid the potential for hepatotoxicity.

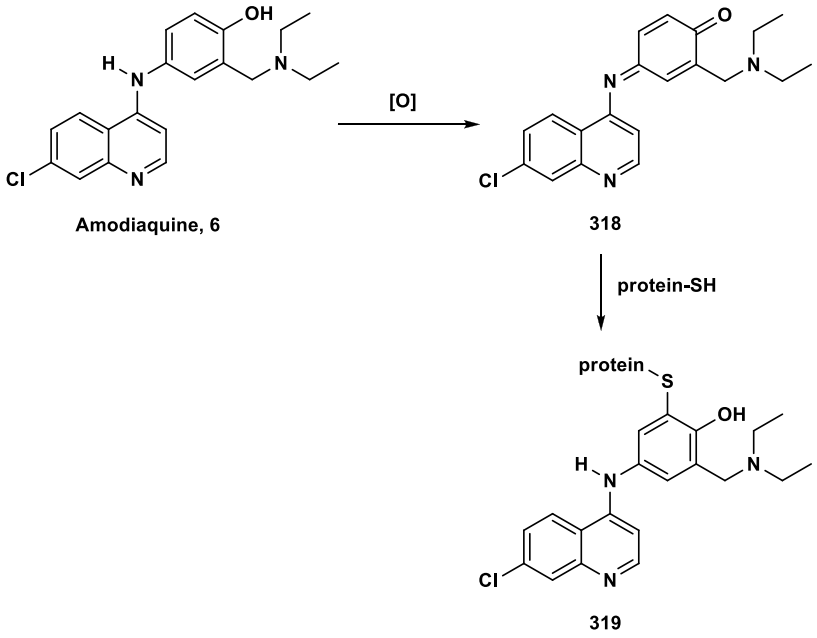


Figure 1.58 Amodiaquine and its toxic metabolite.

Numerous studies have demonstrated improved metabolic stability of fluorine-containing drugs.[56] Accordingly, replacing the hydroxyl group present in the *p*-aminophenol side chain with the bioisostere fluorine is anticipated to block this undesired biotransformation of the side chain (Figure 1.59).

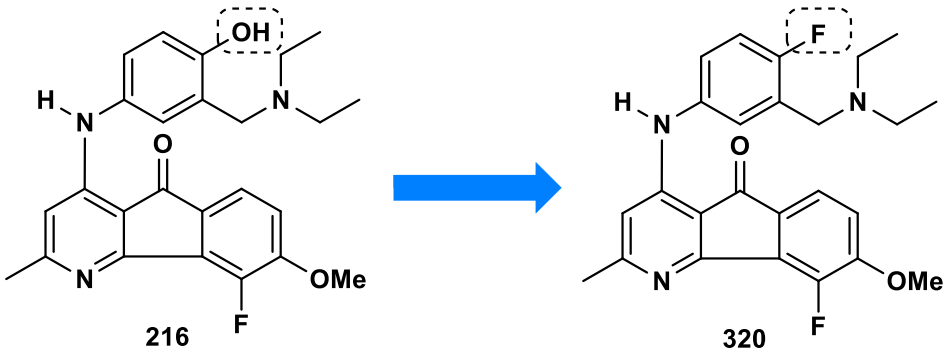


Figure 1.59 Modification of the hybrid molecule containing *p*-aminophenol moiety.

2,4-Diamino-N^{10}-methylpteroic acid **321** (DAMPA, Figure 1.60), a newly developed folate synthesis antagonist, has been proved to be an efficient antimalarial agent.[115] As a mimic of dihydropteroic acid which is a key intermediate in the folic acid metabolic pathway of malarial parasites, DAMPA is able to act as a competitive inhibitor interfering with the biosynthesis of dihydrofolic acid and interrupt the parasites' *de novo* synthesis of dTMP. DAMPA exhibits a high activity against both pyrimethamine-sensitive *Plasmodium falciparum* (M24) and pyrimethamine-resistant *Plasmodium falciparum* (V1/S).[115]

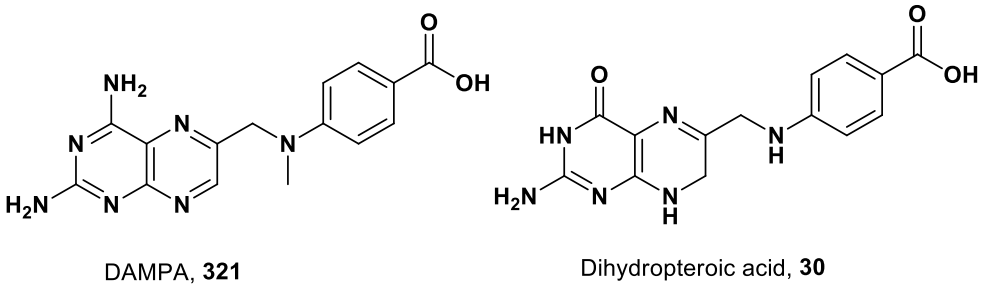
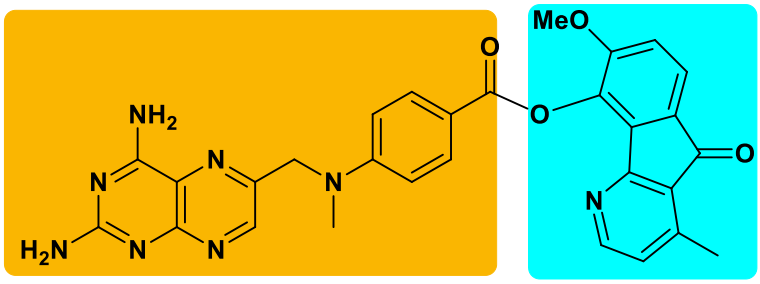


Figure 1.60 Structures of DAMPA and dihydropteroic acid.

The presence of the carboxyl group in DAMPA affords a potential site for appending additional fragments. Accordingly, it is planned to synthesise the prodrug-like dual-acting molecule **322** conjugating DAMPA with an azofluorenone (Figure 1.61). The ester linker connecting the two moieties is expected to be cleaved by esterase

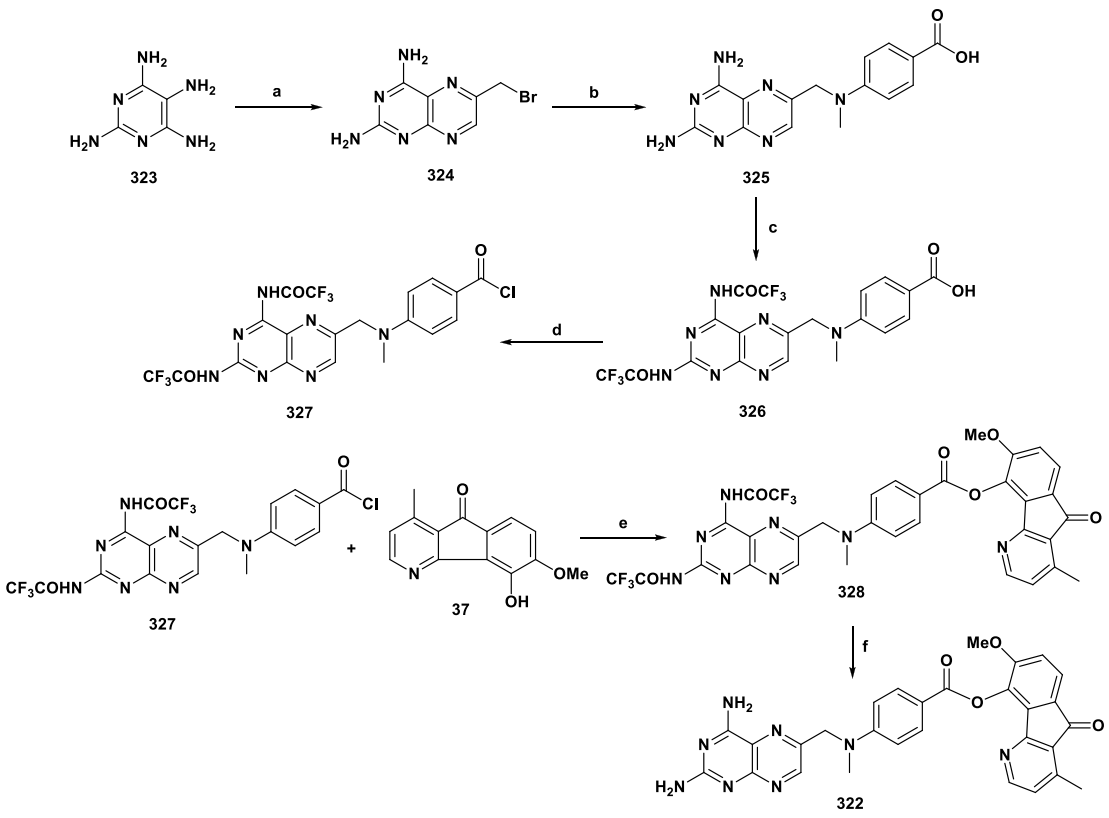
enzymes to release two molecules that would act independently.[116] It is anticipated that the fusion of the two components towards two independent targets would bring about a synergic effect in antiplasmodial action.



322

Figure 1.61 Design of hybrid molecule **322**.

The synthesis of dual-acting molecule **322** will be achieved by the condensation between alkaloid **37** and the acyl chloride **327** derived from DAMPA (Scheme 1.63). DAMPA **321** can be prepared from the commercially available 2,4,5,6-tetraaminopyrimidine *via* an annelation followed by nucleophilic substitution.[117] Protection of the two amino groups will be necessary before activation of the carboxyl group by conversion to the corresponding acyl chloride **327**.[119] The condensation of the two moieties will be achieved in the presence of pyridine.[118] The deprotection of the two amino groups under basic conditions will afford the desired hybrid molecule **322**.[119]



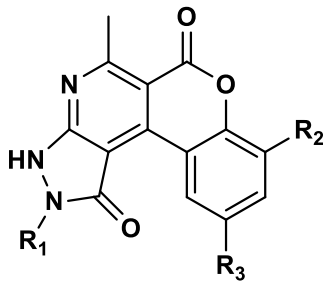
Scheme 1.63 *Reagents and conditions*: (a) 1,1,3-tribromoacetone, HCl aq, EtOH, 50 °C; (b) 4-(methylamino)benzoic acid, NaOH, 40 °C; (c) $(CF_3CO)_2O$, Py, CH_2Cl_2, rt; (d) $SOCl_2$, reflux; (e) Py, CH_2Cl_2, rt; (f) K_2CO_3, MeOH, H_2O, reflux.

CHAPTER 2

One-pot Synthesis of Pyrido[3,2-*c*]coumarins

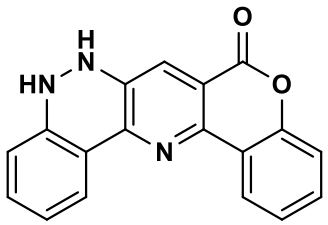
2.1 Introduction and Background

Coumarin and its derivatives are a significant class of oxygen-containing heterocyclic compounds.[122] The coumarin moiety is present in a number of molecules with high biological activity, such as nonpeptidic HIV protease inhibitors,[123] and tyrosine kinase inhibitors.[124] Pyrido-fused coumarins have also been reported to possess significant biological activity. For example, compounds **331** and **332** containing a pyridocoumarin moiety exhibit inhibitory activity towards *Staphylococcus epidermidis*.[125] Polycyclic compound **333** have been proved to possess antituberculosis potency, as it is active against H37Rv strain of *Mycobacterium tuberculosis*.[126]



331, R_1=Ph, R_2=Cl, R_3=Cl

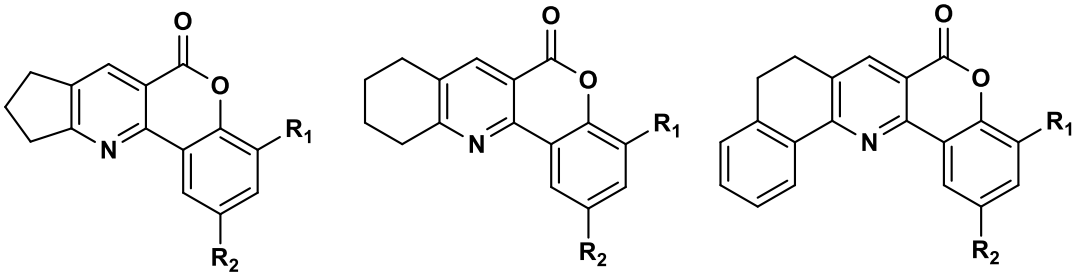
332, R_1=2,5-di-Cl-Ph, R_2=OMe, R_3=H



333

Figure 2.1 Pyridocoumarin derivatives with biological activities.

Recently, a series of polycyclic analogues of pyrido[3,2-*c*]coumarin have been synthesised (Figure 2.2), among which compounds **334**, **335**, **337**, **338**, **340** and **341** display good inhibitory activity against Gram-positive (*Staphylococcus aureus* and *Streptococcus pyogenes*) and Gram-negative (*Pseudomonas phaseolicola* and *Pseudomonas fluorescens*) bacteria, while compounds **334**, **336**, **339**, **341** and **342** show moderate antifungal activity against *Fusarium oxysporum* and *Aspergillus niger*.[127]



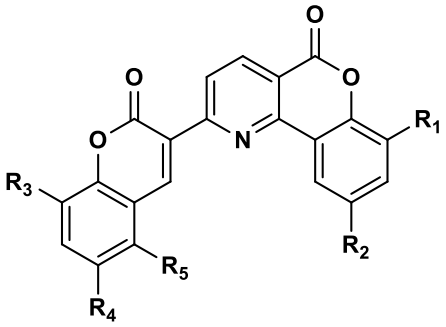
334, R_1=Me, R_2=H
335, R_1=H, R_2=Cl
336, R_1=H, R_2=Me

337, R_1=Me, R_2=H
338, R_1=H, R_2=Cl
339, R_1=H, R_2=H

340, R_1=Me, R_2=H
341, R_1=H, R_2=Cl
342, R_1=H, R_2=H

Figure 2.2 Pyrido[3,2-*c*]coumarin analogues with antibacterial and antifungal activity.

In addition, compounds **343-347** (Figure 2.3) have been proved to exhibit significant antibacterial activity towards *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* as well as *Salmonella typhimurium*.[128] Compounds **343**, **344** and **346** display antifungal activity against *Aspergillus niger*, while compound **347** is active against *Candida albicans.*

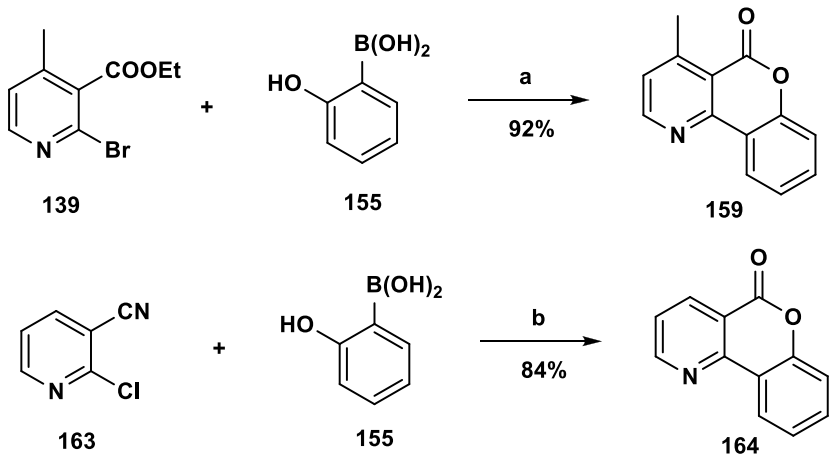


343, R_1=H, R_2=H, R_3=H, R_4=H, R_5=H
344, R_1=H, R_2=H, R_3=OMe, R_4=H, R_5=H
345, R_1=H, R_2=H, R_3=H, R_4, R_5=Benzo
346, R_1=H, R_2=Me, R_3=H, R_4=H, R_5=H
347, R_1=H, R_2=Me, R_3=OMe, R_4=H, R_5=H

Figure 2.3 Pyrido[3,2-*c*]coumarin derivatives with antimicrobial activity.

Owing to the significant biological activities, the synthesis of pyrido-fused coumarins has remained an active subject of interest. As described in Chapter One, an efficient one-pot synthesis of pyrido[3,2-*c*]coumarins based on nicotinates or nicotinonitriles was discovered by us (Scheme 2.1). Given the significant biological properties of pyrido[3,2-*c*]coumarins, it would be of value to develop this one-pot synthesis of

pyrido[3,2-*c*]coumarin analogues.



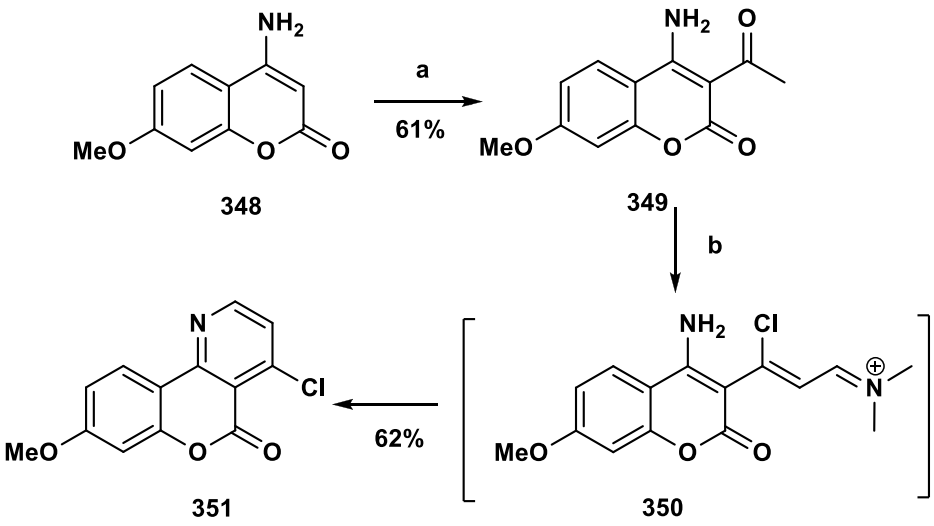
Scheme 2.1 *Reagents and conditions*: (a) $Pd(PPh_3)_4$, THF/H_2O, 2 M Na_2CO_3 aq, 80 °C; (b) $Pd(PPh_3)_4$, DME/H_2O, 2 M K_2CO_3 aq, 80 °C.

2.2 Review of Synthetic Methods for Pyrido[3,2-*c*]coumarins

Previous methods for the synthesis of pyrido[3,2-*c*]coumarins can be divided into four main classes, including cyclisation of substituted coumarins, cyclisation of substituted arylpyridines, condensation of phenols with piperidones, and condensation of salicylic aldehydes with reactive methylene compounds in the presence of ammonia.

2.2.1 Cyclisation of Substituted Coumarin Analogues

Heber developed a synthetic method for pyrido[3,2-*c*]coumarin analogues starting from aminocoumarin (Scheme 2.2).[129] Vilsmeier-Haack acetylation of aminocoumarin **348** afforded ketone **349** in a yield of 61%. Under the conditions of Vilsmeier reaction, phosphorus oxychloride-mediated chlorination of the enol derived from ketone **349** is achieved. Subsequent nucleophilic attack to the Vilsmeier reagent generated *in situ* affords β-chlorovinyliminium salt **350**, which underwent a nucleophilic intramolecular cyclisation followed by the elimination of dimethylamine, fulfilling the annelation and affording the target pyrido[3,2-*c*]coumarin **351** in 62% yield.



Scheme 2.2 *Reagents and conditions*: (a) $POCl_3$, DMA, rt; (b) $POCl_3$, DMF, 45 °C.

Vilsmeier reagent **356**, the reactive iminium salt which plays a crucial role in this synthetic protocol, is formed through the reaction of amide with phosphorus oxychloride (Figure 2.4).

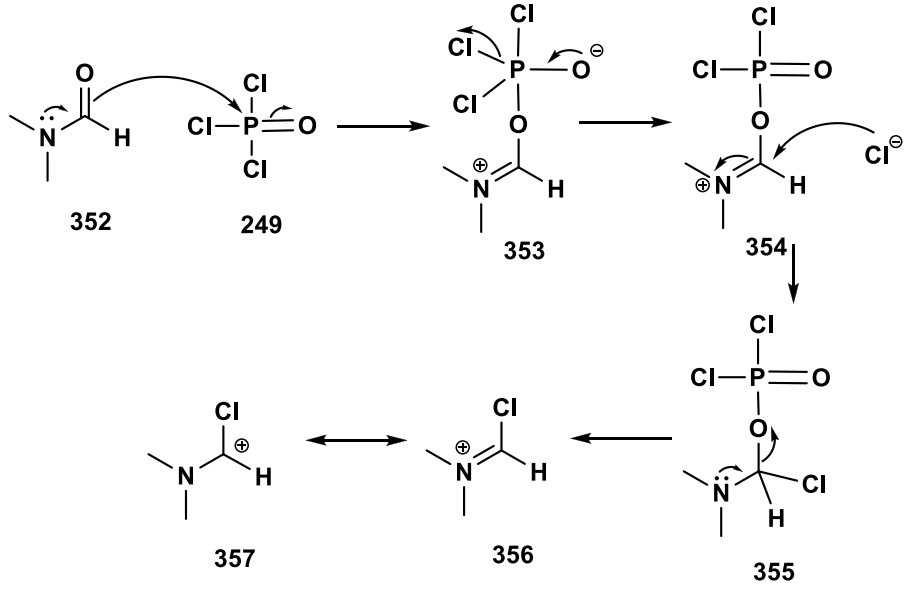
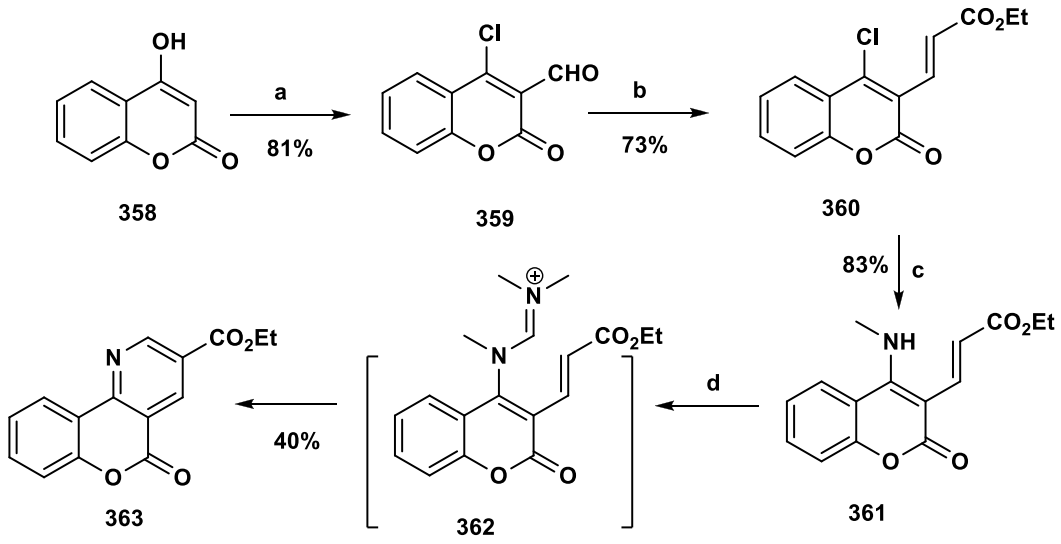


Figure 2.4 Formation of Vilsmeier reagent **356**.

The initial Vilsmeier-Haack formylation of the hydroxycoumarin **358** gave aldehyde **359**.[130] The Wittig reaction of **359** afforded vinylcoumarin **360** in 73% yield. Methylaminocoumarin **361** derived from **360** was then subjected to Vilsmeier reaction

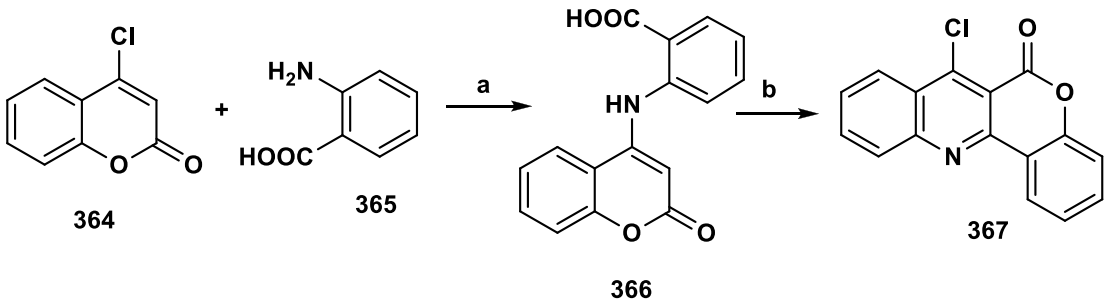
to provide pyrido[3,2-*c*]coumarin **363** in 40% yield. This cyclisation proceeds *via* the intermediate dimethyliminium salt **362**, generated by the nucleophilic attack of 4-methylamino group to the Vilsmeier reagent. The nucleophilic attack of chloride ion to *N*-alky group in **362** provokes the intramolecular cyclisation. With the elimination of dimethyliminium, the ring closure is achieved to give the target pyrido[3,2-*c*]coumarin **363**. However, the low yield (40%) of cyclisation limits the wide application of this method.



Scheme 2.3 *Reagents and conditions*: (a) $POCl_3$, DMF, 60 °C; (b) $Ph_3P{=}CHCO_2Et$, DMF, 0 °C; (c) $MeNH_2$, EtOH, rt; (d) $POCl_3$, DMF, 90 °C.

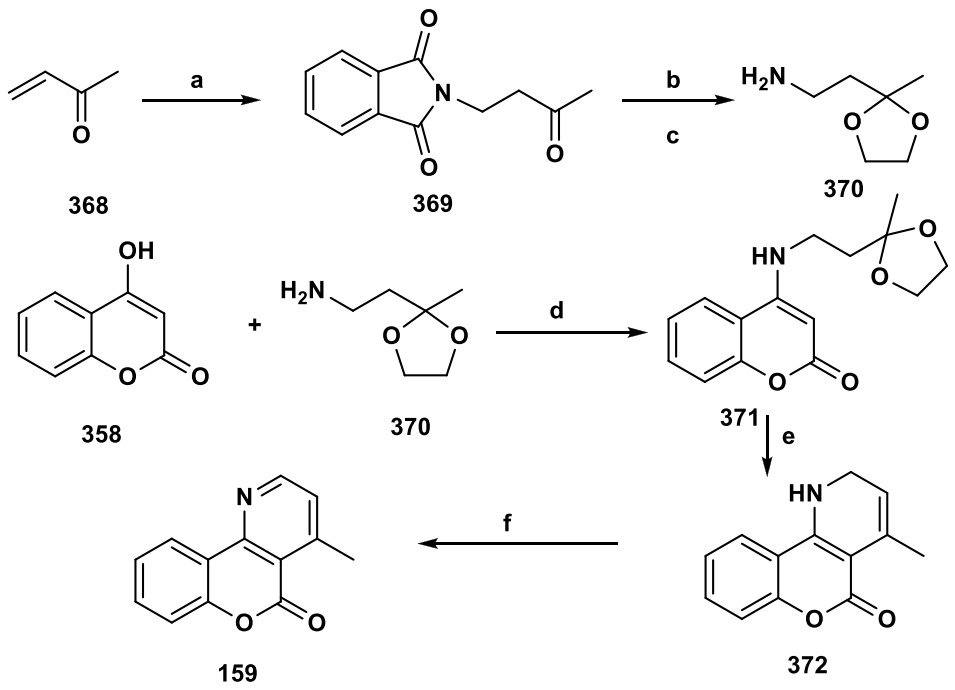
Different from the previous method utilising Vilsmeier-Haack reaction to fulfil the annelation, Mulwad developed an alternative strategy employing intramolecular Friedel-Crafts acylation to achieve the cyclisation (Scheme 2.4).[131] Firstly, 4-chlorocoumarin **364** was treated with anthranilic acid **365** in the presence of copper(I) chloride and triethylamine at 160 °C for 22 hours, fulfilling the Ullmann condensation in 45% yield. Further treatment of condensation product **366** with phosphorus oxychloride at 110 °C for 3 hours afforded the desired product **367** in a yield of 80%. This cyclisation involves three major steps. Carboxylic acid **366** is initially activated by the conversion to acyl chloride mediated by phosphorus oxychloride, which readily

participates in the intramolecular Friedel-Crafts acylation. Subsequent chlorination is achieved by phosphorus oxychloride to provide the target product pyrido[3,2-*c*]coumarin analogue **367**. However, the requirement for long-time harsh conditions (160 °C) and the low yield (45%) of Ullmann condensation are the major defects limiting the wide application of this protocol.



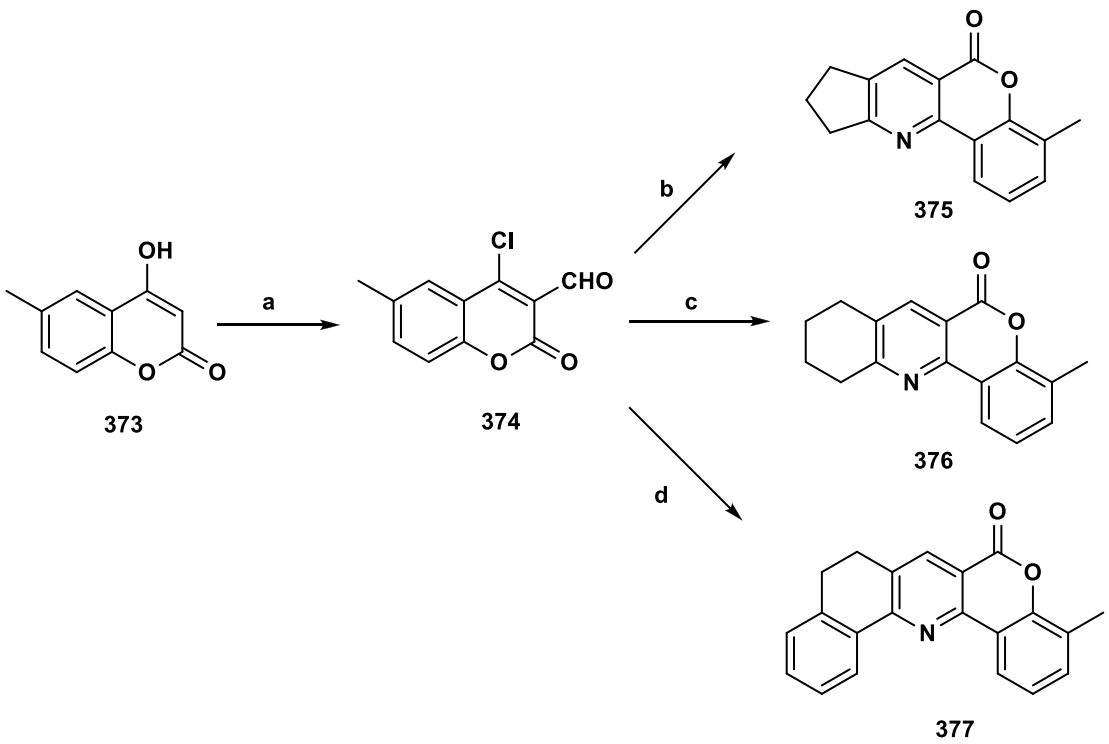
Scheme 2.4 *Reagents and conditions*: (a) CuCl, Et_3N, DMF, 160 °C, 22 h; (b) $POCl_3$, 110 °C, 3 h.

The key building block 2-(2-methyl-1,3-dioxolan-2-yl)ethanamine **370** was prepared from α, β-unsaturated ketones **368** following a three-step synthetic route (Scheme 2.5).[132] Condensation of hydroxycoumarin **358** with amine **370** gave the corresponding aminocoumarin **371**, which underwent an intramolecular cyclisation catalysed by boron trifluoride. Oxidation with DDQ afforded 4-methylpyrido[3,2-*c*]coumarin **159**. The overall yield for this synthetic route is 45%. However, the lengthy multi-step sequences and the utilisation of toxic hydrazine[133] rendered this method unattractive.



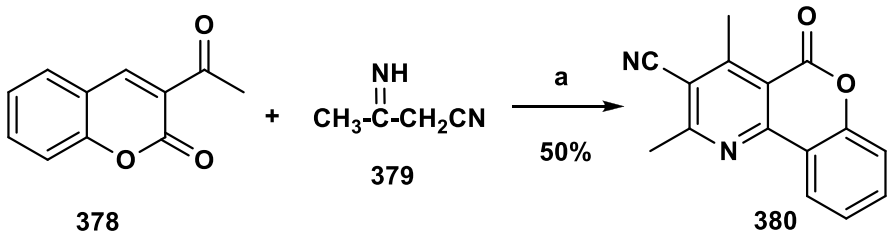
Scheme 2.5 *Reagents and conditions*: (a) phthalimide, trimethylbenzylammonium hydroxide, EtOAc, reflux; (b) $HOCH_2CH_2OH$, TsOH, toluene, reflux; (c) NH_2NH_2, MeOH, reflux; (d) camphorsulfonic acid, toluene, Dean-Stark, reflux, 6h; (e) $BF_3{\cdot}Et_2O$, toluene, reflux; (f) DDQ, CH_2Cl_2, rt,.

The formylation of hydroxycoumarin **373** with Vilsmeier reagent furnished carbaldehyde **374** in 78% yield (Scheme 2.6).[127] Subsequent annelations were achieved by the reactions of coumarin aldehyde **374** with different cyclic ketones in the presence of ammonium acetate in glacial acetic acid, affording the desired polycyclic analogues of pyrido[3,2-*c*]coumarin in 52-58% yields. This protocol provides a good approach to the preparation of polycyclic pyrido[3,2-*c*]coumarins. However, the comparatively low yield of the annelation is a disadvantage limiting the wide ustilisation of this method.



Scheme 2.6 *Reagents and conditions*: (a) $POCl_3$, DMF, 70 °C; (b) cyclopentanone, NH_4OAc, AcOH, reflux; (c) cyclohexanone, NH_4OAc, AcOH, reflux; (d) α-tetralone, NH_4OAc, AcOH, reflux.

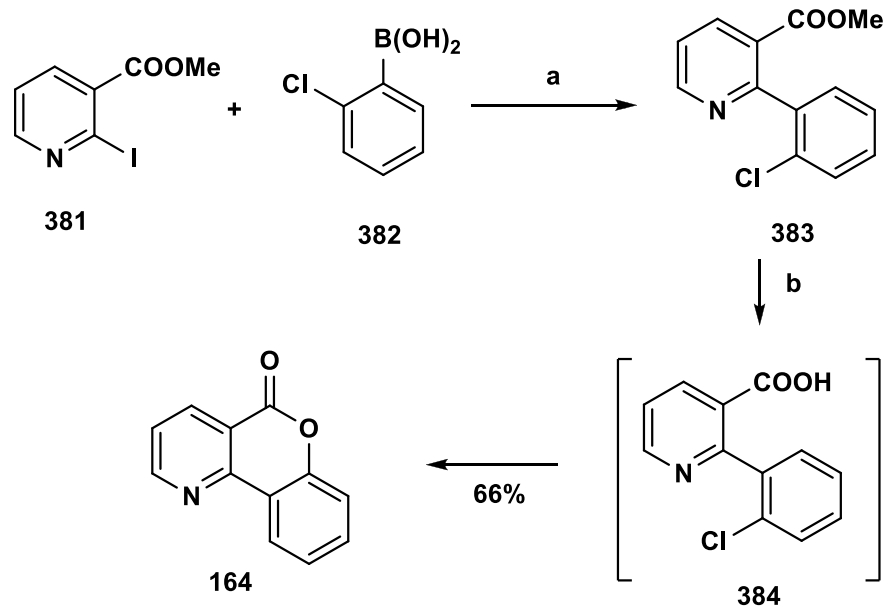
By employing 3-acetyl coumarin derivative **378** as the starting material, Tamam developed a concise synthetic route for pyrido[3,2-*c*]coumarin analogues (Scheme 2.7).[134] Refluxing 3-acetylcoumarin **378** and 3-iminobutyronitrile **379** in the presence of acetic acid in dry benzene furnished the substituted pyrido[3,2-*c*]coumarin **380** in 50% yield. The nucleophilic addition of the highly reactive methylene in 3-iminobutyronitrile **379** to the 3-acetyl group to produces the cyclisation precursor, which undergoes the intramolecular Michael addition of the amino group and subsequent aromatisation, affording the pyrido[3,2-*c*]coumarin **380**. The major advantage of this protocol is the conciseness of the synthetic route. However, the need for toxic benzene and the low yield (50%) overshadow its merit.



Scheme 2.7 *Reagents and conditions*: (a) AcOH, benzene, reflux, Dean-Stark apparatus.

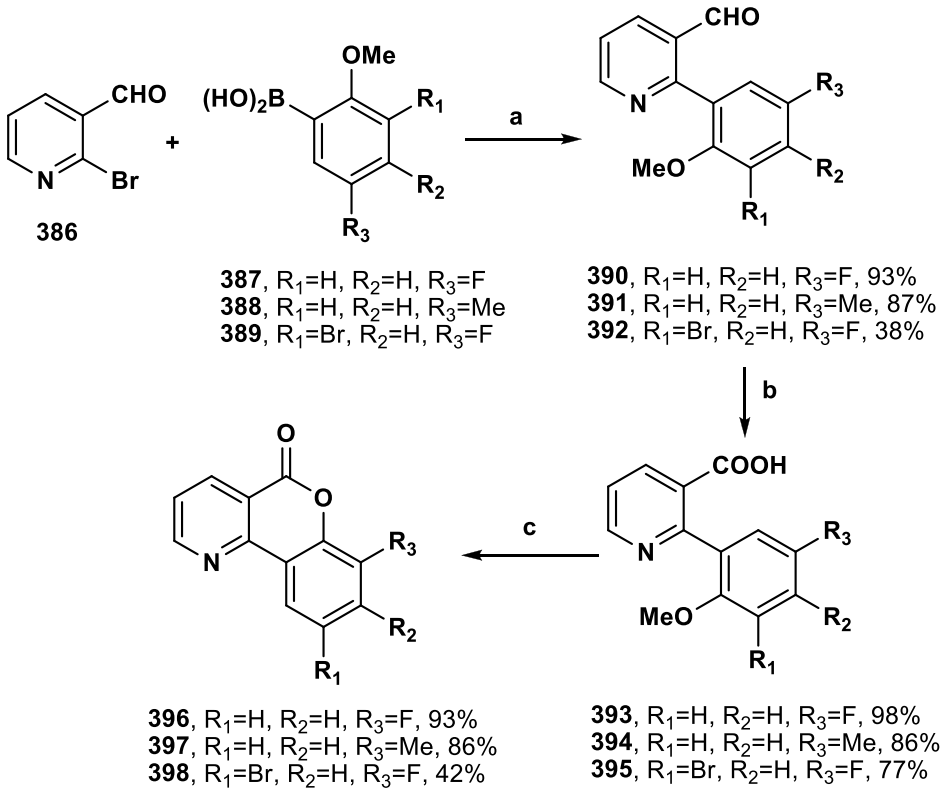
2.2.2 Cyclisation of Substituted Arylpyridines

Recently, a novel method based on the Cu(I)-mediated carbon-oxygen bond formation was reported by Nealmongkol (Scheme 2.8).[135] The Suzuki cross-coupling between 2-iodonicotinate **381** and 2-chlorophenlboronic acid **382** gave cyclisation precursor **383**. The lactonisation was accomplished in the presence of caesium carbonate, copper(I)-thiophene-2-carboxylate (CuTC) and *N,N,N',N'*-tetramethylethylenediamine (TMEDA) in deionised water at 300 °C under microwave irradiation. The desired pyrido[3,2-*c*]coumarin **164** was obtained in 66% yield.



Scheme 2.8 *Reagents and conditions*: (a) $Pd(PPh_3)_4$, 10% Na_2CO_3 solution, toluene, EtOH, reflux; (b)CuTC, Cs_2CO_3. TMEDA, deionised water, microwave irradiation, 300 °C.

The Suzuki cross-coupling of aldehyde **386** with boronic acids **387**-**389** furnished azabiaryls **390**-**392**, which underwent oxidation by sodium chlorite to provide carboxylic acids **393**-**395**. Treatment with boron tribromide achieved the cleavage of methoxy group, and simultaneous cyclisation finally afforded the pyrido[3,2-*c*]coumarins **396**-**398** in moderate to excellent yields (42-93%).[136]

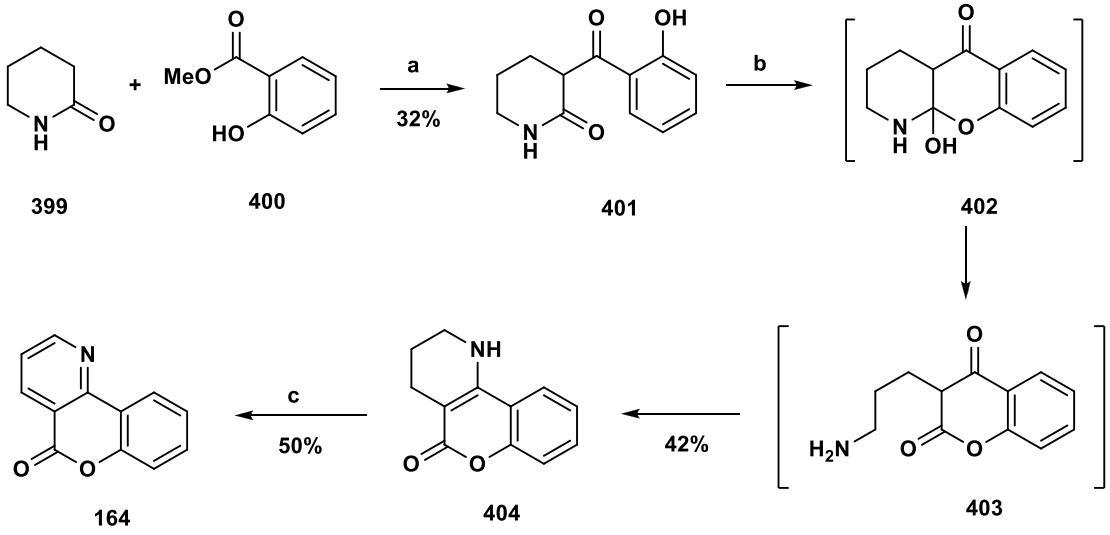


Scheme 2.9 *Reagents and conditions*: (a) $Pd(PPh_3)_4$, K_2CO_3, DMF, 80 °C; (b) $NaClO_2$, NaH_2PO_4, THF, *t*-BuOH, H_2O, rt; (c) BBr_3, CH_2Cl_2, rt.

2.2.3 Condensation of Phenols with Piperidones

Eiden developed a synthetic route initiating from the condensation of piperidin-2-one and 2-hydroxybenzoic acid methyl ester (Scheme 2.10).[137] The intramolecular nucleophilic addition of **401** yielded intermediate **402**, which underwent a ring opening

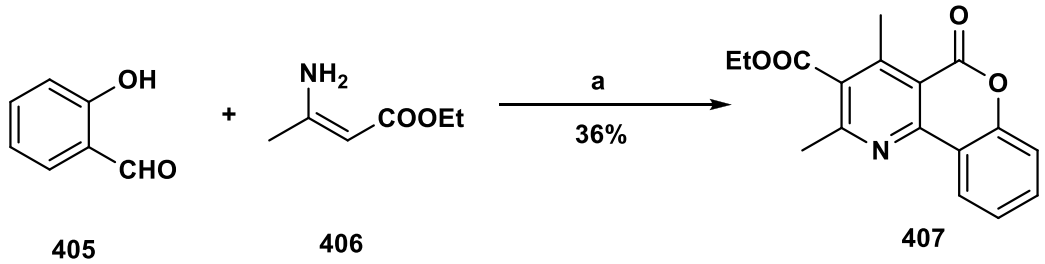
followed by a second intramolecular nucleophilic addition to afford **404**. The cyclisation product **404** was oxidised by DDQ to provide pyrido[3,2-*c*]coumarin **164**. The overall yield of this synthetic route is only 14%.



Scheme 2.10 *Reagents and conditions*: (a) NaH, dioxane, reflux, 18 h; (b) pyridine hydrochloride, blow lamp heating, 2 h; (c) DDQ, MeOH, reflux, 24 h.

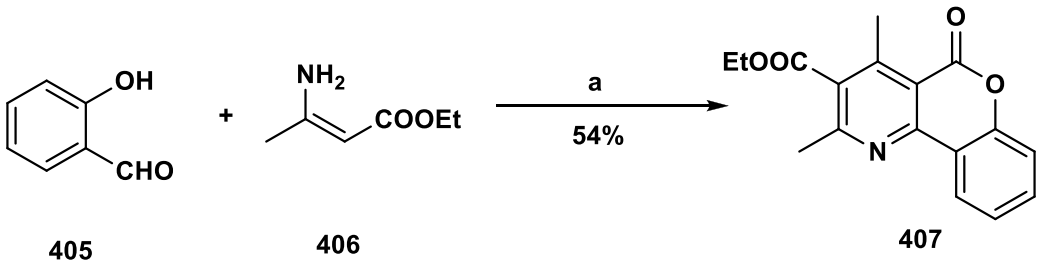
2.2.4 Condensation of Salicylic Aldehydes with Reactive Methylene Compounds in the Presence of Ammonia

Readily available derivatives of salicylic aldehyde can be used to construct pyridocoumarin polycycles with [3,2-*c*] linkages. Condensation of salicylaldehyde **405** and aminocrotonate **406** was performed in acetic acid at room temperature for 4 days, obtaining the pyrido[3,2-*c*]coumarin **407** in a yield of 36% (Scheme 2.11).[138]



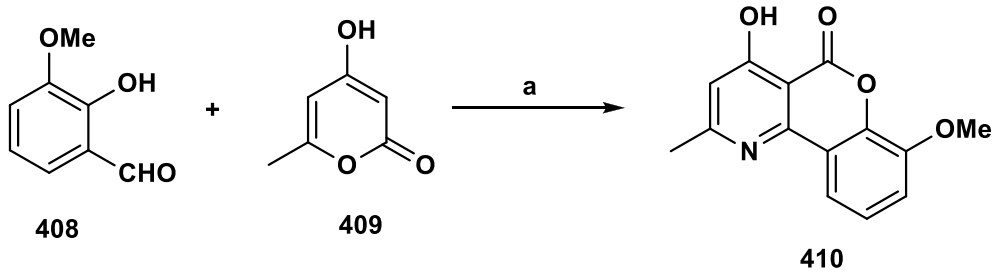
Scheme 2.11 *Reagents and conditions*: (a) CH_3COOH, rt, 4 d.

A recent improved procedure of this protocol was developed by Navarrete-Encina by raising the reaction temperature to 60 °C (Scheme 2.12). Although the time for the reaction was greatly shortened to 5 hours, the yield was still poor 54%.[139]



Scheme 2.12 *Reagents and conditions*: (a) CH_3COOH, 60 ºC, 5 h.

In the presence of ammonium acetate which acted as nitrogen source, condensation of 2-hydroxy-3-methoxybenzaldehyde **408** with 4-hydroxy-6-methyl-2*H*-pyran-2-one **409** in acetic acid afforded the tricyclic product **410** in a low yield of 32% (Scheme 2.13).[140]



Scheme 2.13 *Reagents and conditions*: (a) NH_4OAc, AcOH, reflux

In this process, pyranone **409** serves as the active methylene reactant. The Knoevenagel condensation of salicylic aldehyde derivative **408** with pyranone **409** gives intermediate **411**, whose pyranone moiety undergoes a ring opening reaction to yield acetoacetylcoumarin **412**, which is then converted into enamine **413**. Subsequent intramolecular Michael addition achieves cyclisation and constructing the tricyclic ring system. The final air oxidation and tautomerism provides target pyrido[3,2-*c*]coumarin

410 (Figure 2.5).

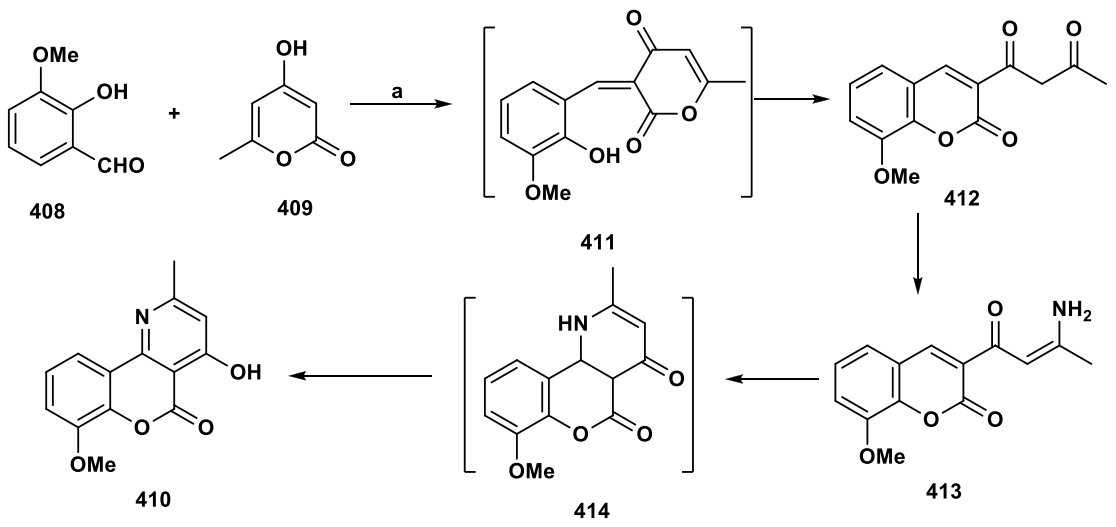
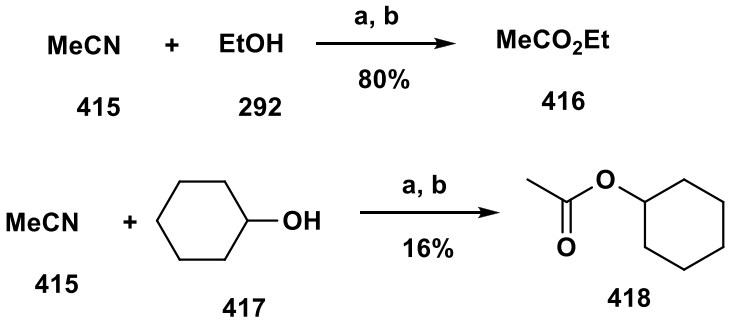


Figure 2.5 Formation of pyrido[3,2-*c*]coumarin **410**.

The survey of the literature reveals that most of the current methods for the synthesis of pyrido[3,2-*c*]coumarins have remarkable defects, such as lengthy synthetic routes, long reaction times, low overall yields or utilisation of toxic reagents. Compared with these methods, our protocol possesses significant advantages. Not only is our synthetic route more concise, but also the yield is improved. The reactants for this method are also widely commercially available and of low-toxicity. More importantly, the one-step reaction is conducted under milder reaction conditions. These milder conditions possess a high functional-group compatibility, affording an ideal means for the synthesis of pyrido[3,2-*c*]coumarin analogues with diverse groups, which could be further functionalised.

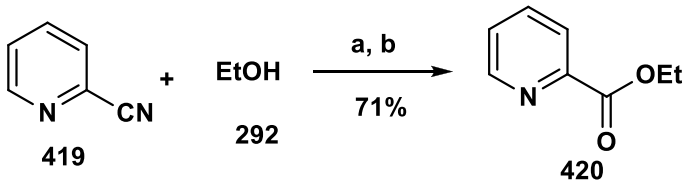
The direct conversion of nitriles to the corresponding carboxylic esters is mainly Bronsted acid or Lewis acid catalysed alcoholysis. The Pinner reaction employing gaseous hydrogen chloride as catalyst exemplifies this conversion.[141] Several improved methods for the Pinner reaction have been developed over the decades. Jiang reported a modified protocol, in which gaseous hydrogen chloride was replaced by ionic liquid

based on a sulfonic acid.[142] 1-Methyl-3-(3-sulphopropyl)-imidazolium hydrogen sulphate ([HSO_3-pmim]HSO_4) was proved to be an efficient catalyst for the esterification of nitriles with alcohols (Scheme 2.14). Nevertheless, this method is only applicable to primary aliphatic alcohols. The utilisation of secondary alcohol **417** resulted in a poor yield of esterification (Scheme 2.14).



Scheme 2.14 *Reagents and conditions*: (a) [HSO_3-pmim]HSO_4, reflux; (b) H_2O, rt.

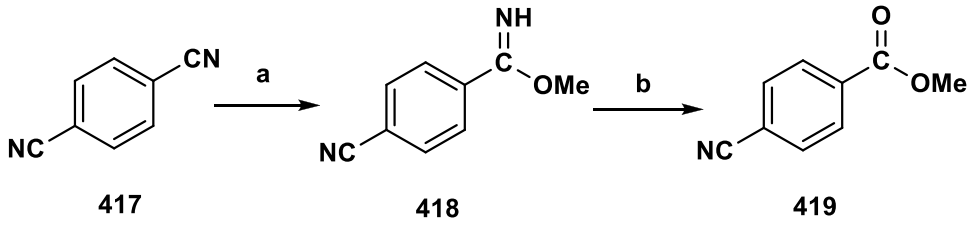
By utilising Lewis acid trimethylsilyl chloride, Luo developed a new method for the acid-catalysed esterification of nitriles (Scheme 2.15).[145] Hydrogen chloride generated *in situ* from the reaction of trimethylsilyl chloride and ethanol serves as the catalyst for this modified Pinner reaction. This method avoids the direct use of gaseous hydrogen chloride, rendering the operation more convenient.



Scheme 2.15 *Reagents and conditions*: (a) TMSCl, EtOH, 50 °C; (b) Na_2CO_3, H_2O, CH_2Cl_2, rt.

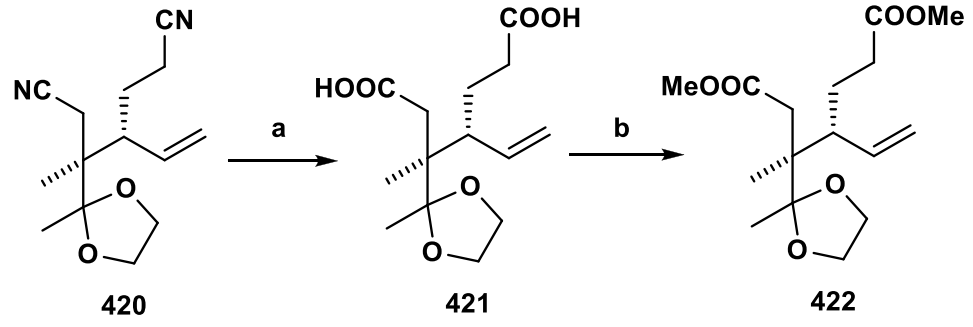
However, the base-catalysed conversions of nitriles to the corresponding carboxylic esters usually require harsh reaction conditions and are not frequently employed in organic synthesis. We've found two examples reported for the conversion of nitriles to

esters under basic conditions. Schaefer employed the strong base sodium methoxide to catalyse this reaction (Scheme 2.16).[146] The treatment of terephthalonitrile **417** with sodium methoxide in methanol under reflux provided an imidate intermediate **418** after 2.5 hours. Subsequent hydrolysis by 12 N hydrochloric acid gave the desired ester **419** only in 61% yield, based on the recovered starting material. Unconverted substrate **417** was recovered in a yield of 24%. The low yield of this esterification and harsh reaction conditions involving the utilisation of strong base and strong acid limits the wide application of this method



Scheme 2.16 *Reagents and conditions*: (a) NaOMe, MeOH, reflux; (b) 12 N hydrochloric acid.

In the synthesis of Vitamin B12, Zutter refluxed nitrile **420** in ethylene glycol and water at 110 °C for 75 h, in the presence of 18.75 equivalents of sodium hydroxide to hydrolyse the cyano groups into carboxyl groups (Scheme 2.17).[147] Subsequent alkylation with methyl iodide in DMF yielded the desired ester **422**.



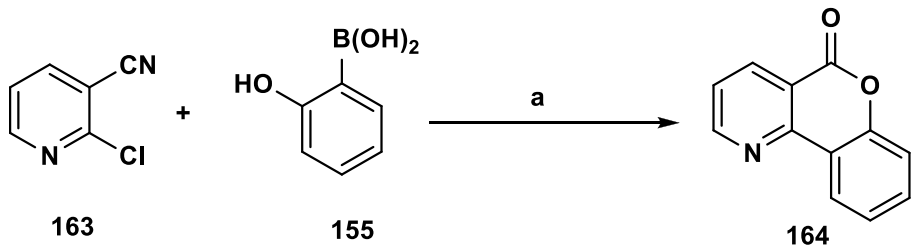
Scheme 2.17 *Reagents and conditions*: (a) NaOH, $HOCH_2CH_2OH$, H_2O, 110 °C; (b) MeI, DMF, rt.

Neither of the two methods described above is a one-step base-catalysed conversion.

Compared with these methods, our protocol requires far milder conditions. The esterification of nitriles under our reaction conditions has not been reported in the literature. Hence, it is of value to investigate this milder one-pot synthesis of pyrido[3,2-*c*]coumarins with nicotinonitriles as the starting core.

2.3 One-pot Synthesis of Pyrido[3,2-*c*]coumarin Analogues under Thermal Conditions

Initially the reaction conditions were optimised for 2-chloronicotinonitrile and 2-hydroxyphenylboronic acid. These were subjected to the Suzuki cross coupling-lactonisation reaction with a range of solvents (Scheme 2.18).



Scheme 2.18 *Reagents and conditions*: (a) 0.1 equiv. $Pd(PPh_3)_4$, 2 M K_2CO_3 aq, solvent, 80 °C, 18 h.

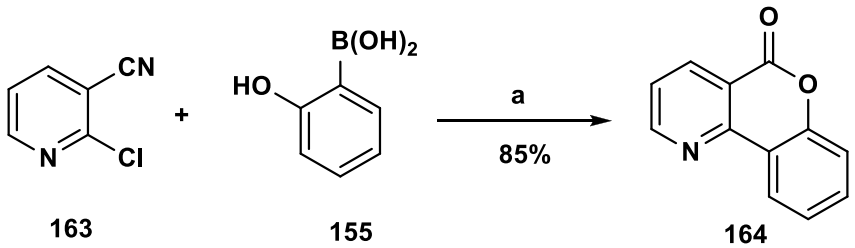
As shown in Table 2.1, pyrido[3,2-*c*]coumarin **164** was afforded in high yields in polar aprotic solvents dimethoxyethane and tetrahydrofuran (Entry 1 and 2). Non-polar aprotic 1,4-dioxane was more efficient as the yield was raised to 89% (Entry 3). Nonetheless, the yield diminished in the case of *N*, *N*-dimethylformamide (Entry 4). This result is attributable to the high polarity of *N*, *N*-dimethylformamide.

Entry	Solvent	Yield (%)	Dielectric Constant (ε)
1	Dimethoxyethane	84	7.3
2	Tetrahydrofuran	81	7.5
3	1,4-Dioxane	89	2.2
4	*N*, *N*-Dimethylformamide	70	38.3

Table 2.1 Screening of different solvents for the one-pot reaction.

As shown in Table 2.1, *N*, *N*-dimethylformamide possesses a much higher dielectric constant than 1,4-dioxane, tetrahydrofuran and dimethoxyethane.[91] When the former three solvents were employed, the reactants were fully dissolved into two phases after the addition of potassium carbonate aqueous solution. However, due to the high dielectric constant of *N*, *N*-dimethylformamide, only one phase was formed after the aqueous solution of base was added, and some reactants precipitated. It could be the poor dissolution of the reactants that hindered the reaction.

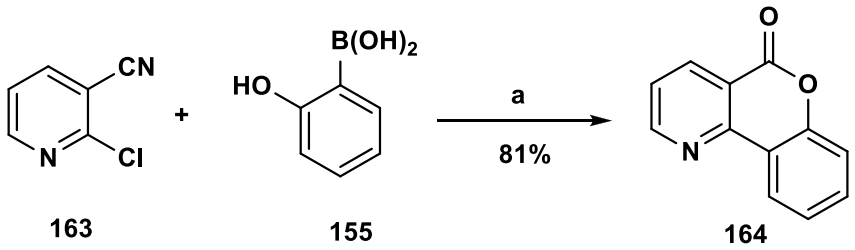
In the presence of tetrakis(triphenylphosphine)palladium(0) and potassium carbonate, the conversion was accomplished in 85% yield when the reaction time was decreased to 12 hours, without a significant decline in yield (Scheme 2.19).



163 155 164

Scheme 2.19 *Reagents and conditions*: (a) 0.1 equiv. $Pd(PPh_3)_4$, 2 M K_2CO_3 aq, 1,4-dioxane/H_2O, 80 °C, 12 h.

Sodium carbonate, a commonly employed base in Suzuki cross-coupling reactions, was also examined. It was demonstrated sodium carbonate solution was similarly efficient to the Suzuki cross coupling-lactonisation, with a slight decrease in yield observed (Scheme 2.20).



Scheme 2.20 *Reagents and conditions*: (a) 0.1 equiv. $Pd(PPh_3)_4$, 2 M Na_2CO_3 aq, 1,4-dioxane/H_2O, 80 °C, 12 h.

To investigate the effect exerted by the quantity of the palladium catalyst on the reaction, another comparative study utilising different equivalents of tetrakis(triphenylphosphine)palladium(0) was conducted. As shown in Table 2.2, no enhancement of yield was observed when the quantity of palladium reagent was raised to 0.2 equivalents (Entry 2). Nevertheless, when the amount of palladium reagent was reduced to 0.03 equivalents, the Suzuki cross coupling-lactonisation was remarkably retarded, requiring more than 48 hours to reach completion (Entry 3).

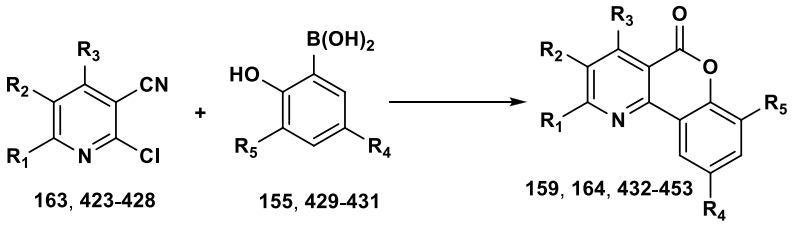
Entry	**Quantity of $Pd(PPh_3)_4$ (equiv.)**	**Reaction Time (h)**	**Yield (%)**
1	0.1	12	85
2	0.2	12	84
3	0.03	48.5	79

Table 2.2 Screening of different quantity of $Pd(PPh_3)_4$.

Consequently, the optimised conditions for this one-pot Suzuki cross coupling-lactonisation are the utilisation of 0.1 equivalent of tetrakis(triphenylphosphine)palladium(0) in the presence of potassium carbonate in 1,4-dioxane and water at 80 °C for 12 hours.

After determining these optimised parameters, a large scale Suzuki cross coupling-lactonisation was attempted using 1 g 2-chloronicotinonitrile. The high yield (88%) for this reaction confirmed the applicability of the method to the large scale synthesis of pyrido[3,2-*c*]coumarins.

To investigate the scope of this protocol, a series of commercially available substrates were subjected to the Suzuki cross coupling-lactonisation reactions. A range of nicotinonitriles bearing electron-withdrawing or electron-donating functional groups at different positions and diverse arylboronic acid bearing electron-withdrawing or electron-donating functional groups were explored. Under the optimal conditions, the anticipated pyrido[3,2-*c*]coumarins were afforded in moderate to excellent yields (Table 2.3), demonstrating the versatility of the protocol. The results indicate that electronic effects influence the one-pot synthesis significantly. In Table 2.3, electron-withdrawing groups are labelled red and electron-donating groups are labelled green. In general, electron-withdrawing groups in the nicotinonitrile substrate promote the reaction (i.e. Entry 5 *versus* Entry 2), while electron-donating groups in the nicotinonitrile render the reaction less efficient (i.e. Entry 21 *versus* Entry 3). In contrast, the electron-withdrawing groups present in the arylboronic acids bring about the opposite effect (i.e. Entry 6 *versus* Entry 4).



Entry	R_1	R_2	R_3	R_4	R_5	Product	Yield (%)
1	H	H	H	H	H	**164**	85
2	H	H	H	Cl	H	**432**	75
3	H	H	H	F	H	**433**	77
4	CF_3	H	H	H	H	**434**	90
5	CF_3	H	H	Cl	H	**435**	81
6	CF_3	H	H	F	H	**436**	78
7	Me	H	H	H	H	**437**	78
8	Me	H	H	Cl	H	**438**	71
9	Me	H	H	F	H	**439**	70
10	H	H	H	H	MeO	**440**	76
11	CF_3	H	H	H	MeO	**441**	80
12	Me	H	H	H	MeO	**442**	72
13	H	Me	H	H	H	**443**	81
14	H	Me	H	Cl	H	**444**	70
15	H	Me	H	F	H	**445**	74
16	H	H	Me	H	H	**159**	76
17	H	H	Me	Cl	H	**446**	69
18	H	H	Me	F	H	**447**	62
19	H	H	MeO	H	H	**448**	72
20	H	H	MeO	Cl	H	**449**	66
21	H	H	MeO	F	H	**450**	60
22	4-F-Ph	H	H	H	H	**451**	80
23	4-F-Ph	H	H	Cl	H	**452**	75
24	4-F-Ph	H	H	F	H	**453**	72

Table 2.3 Synthesis of a library of pyrido[3,2-*c*]coumarins.

These results can be explained by considering the electronic effects exerted by the different groups on the Suzuki cross-coupling catalytic cycle.[58] The oxidative addition of the nicotinonitrile **454** forms a σ-aryl-Pd(II)-Cl species **456** (Figure 2.6). Similar to nucleophilic aromatic substitution reactions, the nucleophilic attack of the palladium reagent affords intermediate **455** with a negative charge on the aromatic ring, which collapses to afford the oxidative addition product **456.**[146] In this process, the presence of electron-withdrawing groups in the halogenated substrate **454** decrease the electron density of the aromatic ring and enhance the electrophilicity, rendering it more vulnerable to nucleophilic attack from the palladium reagent. In addition, the electron-withdrawing groups could stabilise intermediate **455** with a negative charge, significantly facilitating the oxidative addition process (Figure 2.6). Conversely, electron-donating groups present in the halogenated substrate **454** bring about the opposite effects, retarding the oxidative addition.

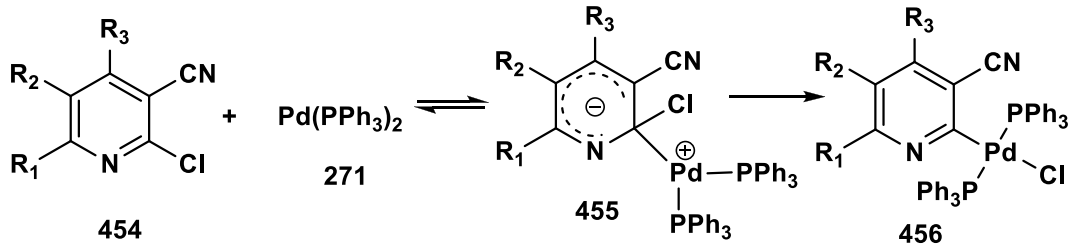


Figure 2.6 Oxidative addition of nicotinonitrile **454**.

In the transmetallation process, the aryl group in boronic acid **458**, serving as a nucleophile, is transferred to the palladium-substrate complex **457**.[58] Electron-withdrawing groups in arylboronic acid **458** diminish its electron density and reduce the nucleophilicity of the aryl group. As a consequence, this nucleophilic transfer of the aryl group is obstructed and the Suzuki cross coupling-lactonisation is retarded (Figure 2.7).

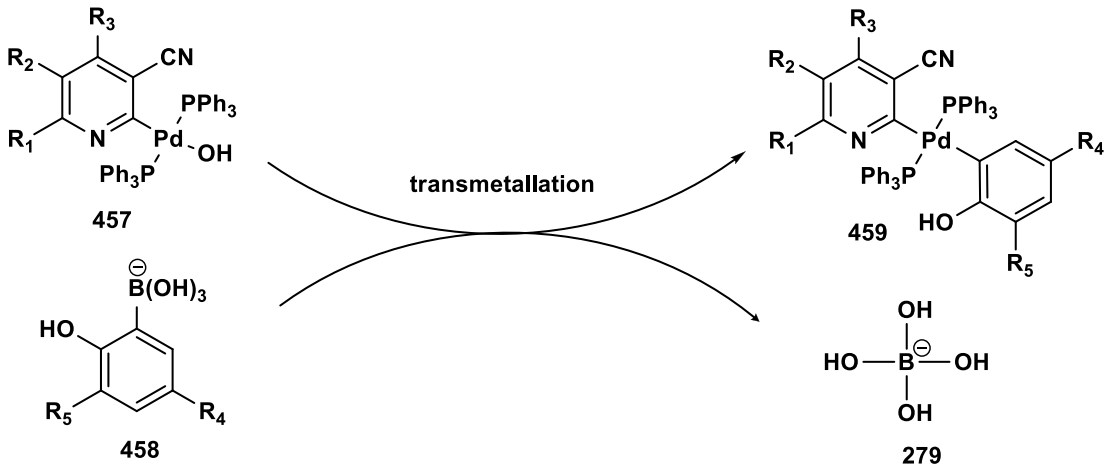
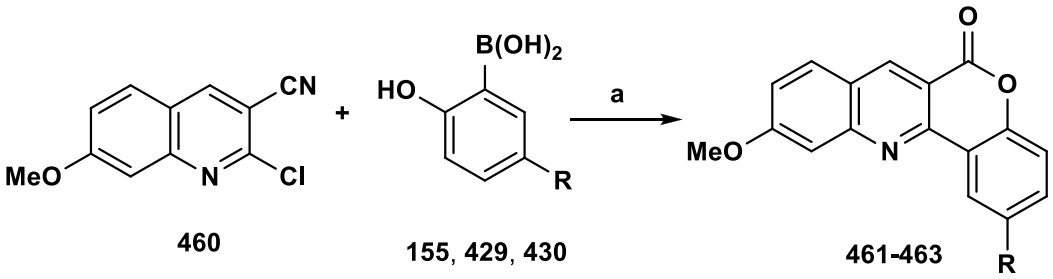


Figure 2.7 Transmetallation in the Suzuki cross-coupling catalytic cycle.

It is noteworthy that in the case of the arylboronic acid substituted with methoxy group which is an electron-donating group, the yields of the reactions were decreased, compared with the corresponding substrates without methoxy group (Entry 10 *versus* Entry 1). This outcome should arise from the unfavourable steric effect of the methoxy group during the subsequent lactonisation.

Chromeno-quinolines, comprising both coumarin and quinoline moieties, have been shown to possess significant biological activity, such as antibacterial activity against *Staphylococcus aureus*.[147] Consequently, it was attempted to prepare 6*H*-chromeno[4,3-*b*]quinolin-6-one analogues using this one-pot strategy (Scheme 2.21). When 2-chloroquinoline-3-carbonitrile substrates were subjected to the optimised reaction conditions, the target products were afforded in moderate to good yields (Table 2.4). The yields obtained for the reactions with quinoline-3-carbonitrile substrates are lower than the reactions of the corresponding nicotinonitrile substrates, which can be attributed to the relative electron richness of the quinoline ring as well as the electron-donating effect of the methoxy group in substrate **460**.

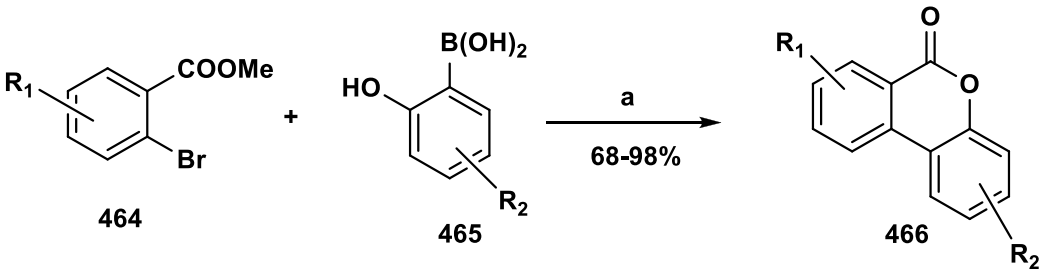


Scheme 2.21 *Reagents and conditions*: (a) $Pd(PPh_3)_4$, 2 M K_2CO_3 aq, 1,4-dioxane/H_2O, 80 °C.

Entry	R	Product	Yiled (%)
1	H	**461**	75
2	Cl	**462**	67
3	F	**463**	62

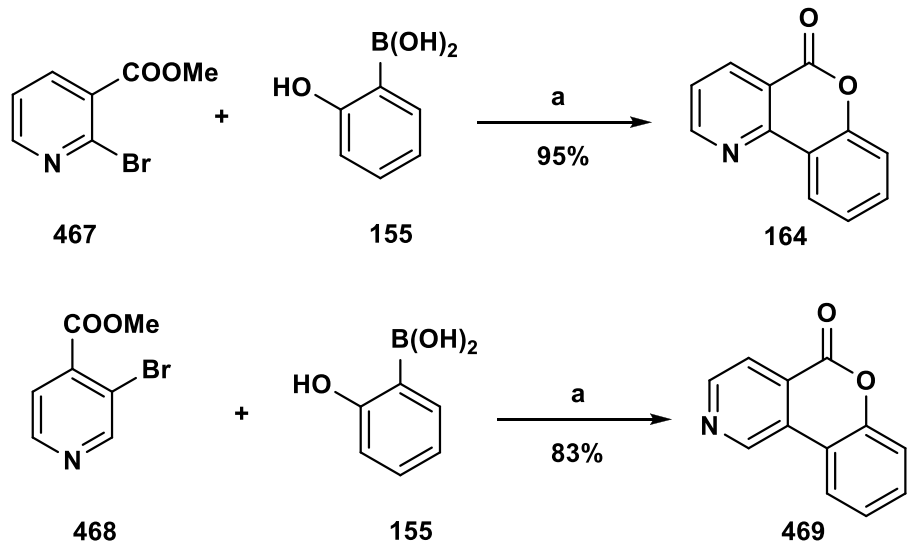
Table 2.4 Preparation of 6*H*-chromeno[4,3-*b*]quinolin-6-one analogues.

During the course of these investigations, we became aware of a report for the microwave-promoted synthesis of dibenzopyranones.[148] By utilising bromophenyl carboxylates **464** and hydroxyarylboronic acids **465**, with caesium carbonate as the base, a series of dibenzopyranones were prepared in moderate to excellent yields under the microwave-heating conditions (Scheme 2.22).



Scheme 2.22 *Reagents and conditions*: (a) $Pd(PPh_3)_4$, Cs_2CO_3, DME/H_2O, MW 125 °C.

Two bromonicotinate substrates were also examined under these conditions (Scheme 2.23). The anticipated pyrido-coumarins were afforded in 95% and 83% yields, respectively.



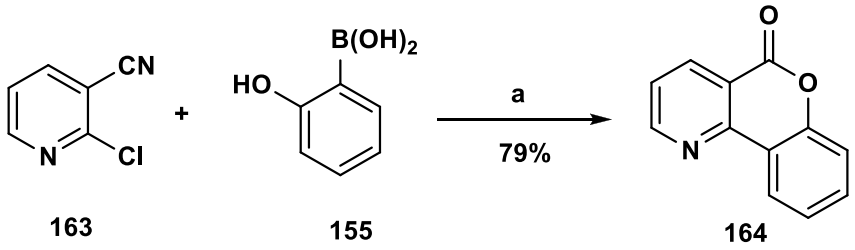
Scheme 2.23 *Reagents and conditions*: (a) $Pd(PPh_3)_4$, Cs_2CO_3, DME/H_2O, MW 125 °C,

Within comparison to this method, our protocol utilises chloronicotinonitriles instead of the bromonicotinate substrates. Chlorinated substrates in the Suzuki cross-coupling reactions are much less reactive than the corresponding brominated substrates,[58] while the chlorinated substrates are far more widely available and much lower in cost than the corresponding brominated substrates. Our research has proved that the less reactive and cheaper chlorinated substrates perform well for the synthesis of pyrido[3,2-*c*]coumarins.

2.4 One-pot Synthesis of Pyrido[3,2-*c*]coumarin Analogues under Microwave-irradiated Conditions

Initially, 2-chloronicotinonitrile and 2-hydroxyphenylboronic acid were subjected to the microwave-heating conditions (Scheme 2.24). In the presence of 0.1 equivalent of tetrakis(triphenylphosphine)palladium(0) and 2 M potassium carbonate solution, pyrido[3,2-*c*]coumarin **164** was afforded in a yield of 79% after microwave heating at 125 °C for 30 min. This demonstrates that the one-pot Suzuki cross coupling-lactonisation of the nicotinonitrile substrate can also be achieved under the

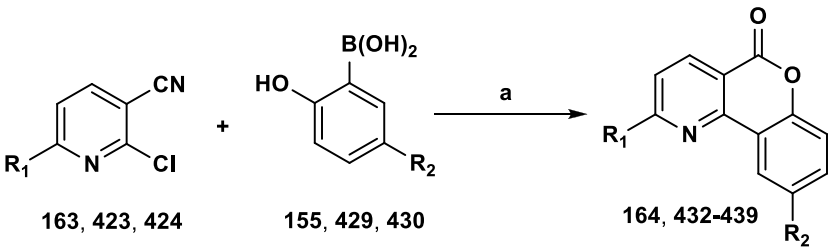
microwave-heating conditions.



Scheme 2.24 *Reagents and conditions*: (a) $Pd(PPh_3)_4$, 2 M K_2CO_3 aq, 1,4-dioxane/H_2O, MW 125 °C, 30 min.

It was observed that the yield of this reaction under microwave-heating conditions was lower than the one under thermal conditions. Taking the difficulty of the base-catalysed alcoholysis of nitriles into account, longer reaction times for the lactonisation of nicotinonitriles under microwave-irradiated conditions may be required. Accordingly, 2-chloronicotinonitrile and 2-hydroxyphenylboronic acid were subjected to the microwave irradiation for 1 hour, resulting in a comparable yield of 80%.

The scope of the microwave-assisted reaction was investigated using a range of nicotinonitriles and arylboronic acids containing electron-withdrawing or electron-donating groups (Scheme 2.25). The target pyrido[3,2-*c*]coumarin analogues were obtained in moderate yields (Table 2.5).



Scheme 2.25 *Reagents and conditions*: (a) $Pd(PPh_3)_4$, 2 M K_2CO_3 aq, 1,4-dioxane/H_2O, MW 125 °C, 30 min.

Entry	R_1	R_2	Product	Yield (%)
1	H	H	**164**	79
2	H	Cl	**432**	76
3	H	F	**433**	66
4	CF_3	H	**434**	80
5	CF_3	Cl	**435**	75
6	CF_3	F	**436**	72
7	Me	H	**437**	74
8	Me	Cl	**438**	63
9	Me	F	**439**	69

Table 2.5 Synthesis of pyrido[3,2-*c*]coumarins under microwave-irradiated conditions

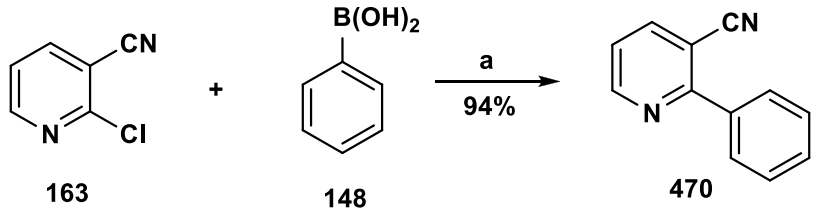
The results from the microwave-assisted reactions are generally consistent with the results under thermal heating conditions. The electron-withdrawing group present in the nicotinonitrile promoted the microwave-assisted one-pot reaction (Entry 6 *versus* Entry 3) and the electron-donating group retarded the reaction (Entry 8 *versus* Entry 2). Conversely, the electron-withdrawing group in the arylboronic acid substrate rendered the yield decreased (Entry 3 *versus* Entry 1).

2.5 Mechanistic Considerations

Since the conversion of nitriles to the corresponding carboxylic esters are predominately acid catalysed, we set out to probe the mechanism of this unusual one-pot synthesis of pyrido[3,2-*c*]coumarins. The key question to be answered is whether this reaction proceeds *via* an amide intermediate.

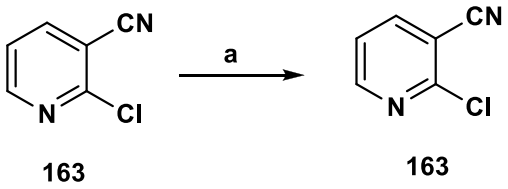
Under the identical thermal reaction conditions developed for the preparation of pyrido[3,2-*c*]coumarins, the treatment of 2-chloronicotinonitrile with phenylboronic

acid afforded the cross-coupling product **470** in an excellent yield (Scheme 2.26).



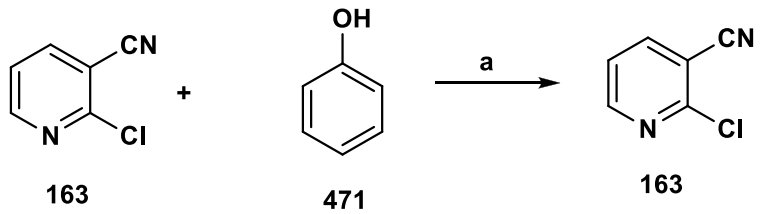
Scheme 2.26 *Reagents and conditions*: (a) $Pd(PPh_3)_4$, 2 M K_2CO_3 aq, 1,4-dioxane/H_2O, 80 °C.

The stability of 2-chloronicotinonitrile was investigated. Stirring in aqueous potassium carbonate solution and 1,4-dioxane at 80 °C resulted in a complete recovery of 2-chloronicotinonitrile after 12 hours (Scheme 2.27)



Scheme 2.27 *Reagents and conditions*: (a) 2 M K_2CO_3 aq, 1,4-dioxane/H_2O, 80 °C.

In addition, the potential reaction of 2-chloronicotinonitrile and phenol was investigated. In the presence of potassium carbonate solution, 2-chloronicotinonitrile and phenol in 1,4-dioxane failed to afford the condensation product, with 2-chloronicotinonitrile substrate being completely recovered after 12 hours (Scheme 2.28).



Scheme 2.28 *Reagents and conditions*: (a) 2 M K_2CO_3 aq, 1,4-dioxane/H_2O, 80 °C.

These observations support the assertion that this one-pot reaction does not proceed *via* an amide intermediate, as the cyano group was not hydrolysed to an amide under these reaction conditions. The model reaction shown in Scheme 2.28 indicates that the intermolecular nucleophilic attack of phenol hydroxyl group to the cyano group cannot occur under the reaction conditions employed, suggesting that the Suzuki cross-coupling reaction occurs prior to the lactonisation. Only the arylpyridine structure derived from the cross-coupling can provide a favourable conformation facilitating the occurrence of intramolecular nucleophilic attack from phenol hydroxyl group to construct the tricyclic ring system.

The proposed mechanism of the cross-coupling reactions is shown in Figure 2.7. Initially, the Suzuki-Miyaura cross-coupling of **163** and **155** yields azabiaryl **471**. Under the basic condition, the phenol hydroxyl group in **471** is deprotonated to form the phenolate anion, whose nucleophilicity is much greater than the phenol. Intramolecular nucleophilic attack from the phenolate anion to the cyano group constructs the tricyclic ring system. Imidate anion **472** takes a proton from water and subsequently undergoes the nucleophilic attack of hydroxide ion, yielding tetrahedral intermediate **474**. After the intramolecular proton transfer, intermediate **55** collapses to afford pyrido[3,2-*c*]coumarin **164**.

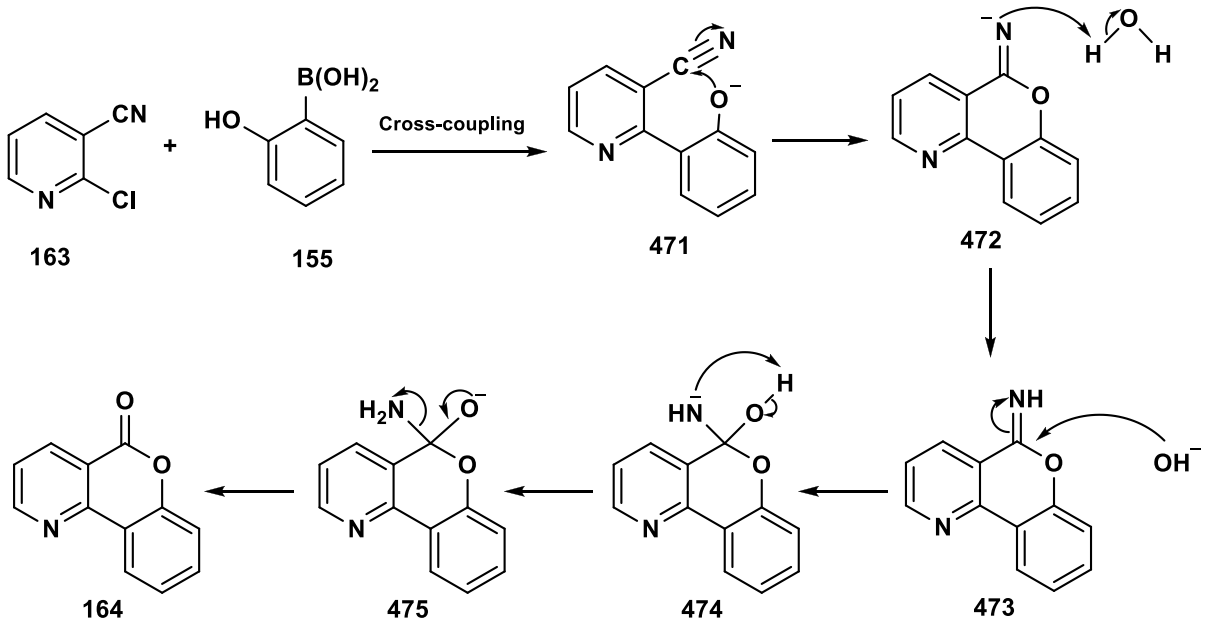


Figure 2.8 Mechanistic proposal for the one-pot synthesis of pyrido[3,2-*c*]coumarins.

2.6 Summary and Conclusions

An efficient one-pot method for the synthesis of pyrido[3,2-*c*]coumarin analogues has been developed. A range of different nicotinonitrile and arylboronic acid substrates have been investigated. Twenty seven pyrido[3,2-*c*]coumarin derivatives have been prepared in moderate to excellent yields under the optimised thermal conditions, which confirms the versatility of this protocol. It has been demonstrated that the electronic effects of the substrates is an important factor influencing the yield of this reaction. Electron-withdrawing groups present in the nicotinonitrile substrates promote this one-pot synthesis, while electron-donating groups in the nicotinonitrile substrates and electron-withdrawing groups in the arylboronic acid substrates retard this reaction. Moreover, microwave-assisted one-pot synthesis of pyrido[3,2-*c*]coumarins has also been investigated. Nine analogues of pyrido[3,2-*c*]coumarin have been prepared in moderate to good yields, confirming that this one-pot synthetic method is also efficient

under microwave-heating conditions. The good yields, concise synthetic route, wide availability of substrates and milder reaction conditions render this one-pot protocol superior to other commonly used methods and make it a feasible approach to the synthesis of pyrido[3,2-*c*]coumarins in gram quantities.

In addition, the mechanism of this cross coupling-lactonisation has been explored. The model reactions performed support the perspective that the Suzuki reaction occurs prior to the lactonisation and this one-pot reaction does not proceed *via* an amide intermediate. A mechanism proposal for this one-pot synthesis of pyrido[3,2-*c*]coumarins has been put forward.

CHAPTER 3

Experimental

3.1 General Experimental Procedures

Infrared (IR) spectra were recorded using a Perkin Elmer FTIR spectrophotometer. Only significant absorptions (ν_{max}) are reported and all absorptions are recorded in wavenumbers (cm^{-1}). Melting points (mp) were measured on Barnstead Electrothermal 9100 apparatus and are uncorrected.

Proton magnetic resonance spectra (^{1}H NMR) were recorded at 400 MHz using a Bruker spectrometer. Chemical shifts (δ_H) are quoted in parts per million (ppm) and are referenced to the residual protonated solvent peak. The order of citation in parentheses is (*i*) number of equivalent nuclei (by integration), (*ii*) multiplicity (s, singlet; d, doublet; t, triplet; q, quartet and m, multiplet), (*iii*) coupling constant (*J*) quoted in Hertz to the nearest 0.1 Hz, (*iv*) assignment. Carbon magnetic resonance spectra (^{13}C NMR) were recorded at 100 MHz using a Bruker spectrometer. Chemical shifts (δ_C) are quoted in parts per million (ppm) and are referenced to the appropriate solvent peak. Fluorine magnetic resonance spectra (^{19}F NMR) were recorded at 376 MHz using a Bruker spectrometer. Chemical shifts (δ_F) are quoted in parts per million (ppm) and are referenced to trifluoroacetic acid as external standard.

Low resolution mass spectra (*m/z*) were recorded using LCQ DECA XP instrument by electrospray ionisation (ESI). Only molecular ions are reported. Accurate masses were

determined using a quadrupole time-of-fight mass (QTOF) spectrometer at King's College London or a Thermofisher LTQ Orbitrap XL instrument at the EPSRC National Mass Spectrometry Facility in Swansea by electrospray ionisation (ESI).

Reactions were carried out under dry, oxygen free nitrogen and all reagents were used as received unless otherwise stated. Microwave-assisted reactions were performed on a Monowave 300 Microwave Synthesis Reactor from Anton Paar. The fractions of light petroleum ether boiling between 40 and 60 °C are referred to as "hexanes". Solvents were evaporated using a Büchi Rotavapor RE111.

Flash Chromatography was carried out using silica gel (Sigma, 230-400 mesh) as the stationary phase. Thin Layer Chromatography was carried out on aluminium plates pre-coated with silica (Merck silica gel 60 F_{254} on aluminium) which was visualised by the quenching of ultraviolet fluorescence (λ_{max}=254nm).

The azafluorenones evaluated for the antiplasmodial activity were analysed by high performance liquid chromatography (HPLC) using three different linear gradients. The purity of the compounds was found to be over 95%. The HPLC analysis was conducted on a Hewlett-Packard 1050 system equipped with an autosampler, a reversed-phase HPLC column (Agilent Zorbax 300Å, C18, 5 μ M, 150 × 2 mm) and a diode array detector (DAD) set to monitor 210-280 nm. The column was developed using three linear gradients:

(i) 0-90% CH_3CN in 0.1%(v/v) trifluoroacetic acid aqueous solution within 20 min (t_{R1});

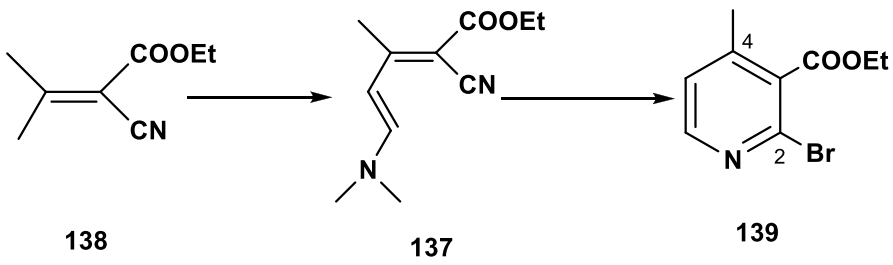
(ii) 0-90% CH_3CN in 0.1%(v/v) trifluoroacetic acid aqueous solution within 30 min (t_{R2});

(iii) 0-50% CH_3CN in 0.1%(v/v) trifluoroacetic acid aqueous solution within 50 min (t_{R3}).

The *in vitro* β-haematin inhibition assays were carried out in Nunc clear, flat-bottom 96-well plates. Centrifugations were carried out in a Beckman Coulter Allegra 25R rotor. The absorbance of the samples was measured at 400 nm or 405 nm on a BMG Labtech POLARstar Optima multiplate reader. Sigmoidal concentration-response curves and the IC_{50} values were acquired from the software of GraphPad Prism v5.0.

3.2 Chemical Synthesis

Ethyl 2-bromo-4-methylnicotinate

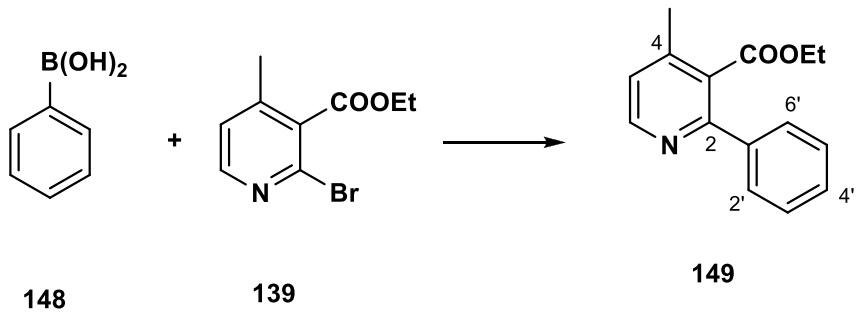


138 **137** **139**

To a solution of ethyl 2-cyano-3-methylbut-2-enoate **138** (11.40 g, 74.42 mmol, 1.0 equiv.) in absolute ethanol (70 mL), *N*,*N*-dimethylformamide dimethyl acetal (9.15 mL, 74.42 mmol, 1.0 equiv.) was added dropwise. After addition, the solution was

heated at reflux for 6 h and then concentrated *in vacuo* to yield crude (*2E,4E*)-ethyl 2-cyano-5-(dimethylamino)-3-methylpenta-2,4-dienoate **137**. Crude **137** was dissolved in acetic acid (50.0 mL) and the mixture was heated at 40 ºC. A solution of 30% HBr-AcOH (91.0 mL) was added dropwise, then the mixture was heated to 55 ºC with stirring. After heating for 45 min, the solution was poured onto ice, neutralised with solid sodium carbonate, and extracted with dichloromethane (4 × 200 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (10.5% ethyl acetate-hexane) afforded ethyl 2-bromo-4-methylnicotinate **139** (10.58 g, 62% over two steps) as a colourless oil; R_f 0.31 (12.5% ethyl acetate-hexane); ν_{max} (neat) 2982, 1728 (C=O), 1579, 1446, 1379, 1273, 1118, 1065, 859 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.19 (1H, d, J 5.2 Hz, H-6), 7.06 (1H, d, J 5.2 Hz, H-5), 4.39 (2H, q, J 7.2 Hz, O-C<u>H</u>$_2$-CH_3), 2.29 (s, 3H, 4-C<u>H</u>$_3$), 1.36 (3H, t, J 7.2 Hz, O-CH_2-C<u>H</u>$_3$); δ_C (100 MHz, $CDCl_3$) 165.0(C=O), 148.5 (C-6), 145.7 (C-4), 137.0 (C-2), 131.9 (C-3), 123.0 (C-5), 60.8 (O-<u>C</u>H_2-CH_3), 17.9 (4-<u>C</u>H_3), 12.6 (O-CH_2-<u>C</u>H_3). *In agreement with published data*.[61]

Ethyl 2-phenyl-4-methylnicotinate

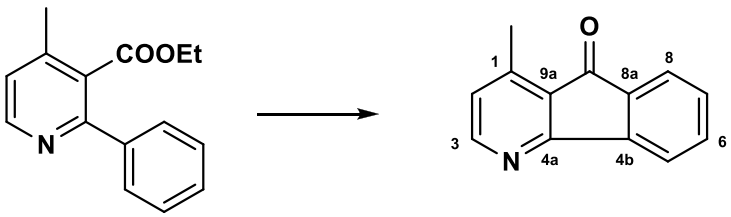


148 **139** **149**

A mixture of **139** (488 mg, 2.00 mmol, 1.0 equiv.), phenylboronic acid **148** (268 mg, 2.20 mmol, 1.1 equiv.), tetrakis(triphenylphosphine)palladium(0) (231 mg, 0.20 mmol, 0.1 equiv.) and tetrahydrofuran (20 mL) was stirred at 80 ºC for 1 h, then sodium

carbonate solution (2 M aqueous solution; 3.0 mL, 6.00 mmol, 3.0 equiv.) was added and the resulting mixture stirred at 80 ºC for 18 h. The mixture was diluted with dichloromethane (100 mL) and water (20 mL). The layers were separated and the organic phase washed with water (20 mL) and brine (20 mL). The combined aqueous phases were extracted with dichloromethane (3 × 100 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (14.3% ethyl acetate-hexane) afforded ethyl 2-phenyl-4-methylnicotinate **149** (425 mg, 88%) as a colourless oil; R_f 0.21 (16.7% ethyl acetate-hexane); ν_{max} (neat) 2981, 1722 (C=O), 1604, 1565, 1286, 1250, 775 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.51 (1H, d, J 5.2 Hz, H-6), 7.52-7.50 (2H, m, H-2', H-6'), 7.37-7.32 (3H, m, H-3', H-5', H-4'), 7.08 (1H, d, J 5.2 Hz, H-5), 4.06 (2H, q, J 7.2 Hz, O-C<u>H</u>$_2$-CH_3), 2.36 (s, 3H, 4-C<u>H</u>$_3$), 0.94 (3H, t, J 7.2 Hz, O-CH_2-C<u>H</u>$_3$); δ_c (100 MHz, $CDCl_3$) 166.5 (C=O), 154.5 (C-6), 147.4 (C-4), 143.5 (C-2), 137.8 (C-1'), 127.1 (C-3), 126.4 (C-3', C-5'), 126.1 (C-2', C-4'), 126.0 (C-6'), 121.5 (C-5), 59.2 (O-<u>C</u>H_2-CH_3), 17.2 (4-<u>C</u>H_3), 11.4 (O-CH_2-<u>C</u>H_3). *In agreement with published data*.[149]

1-Methyl-4-azafluoren-9-one

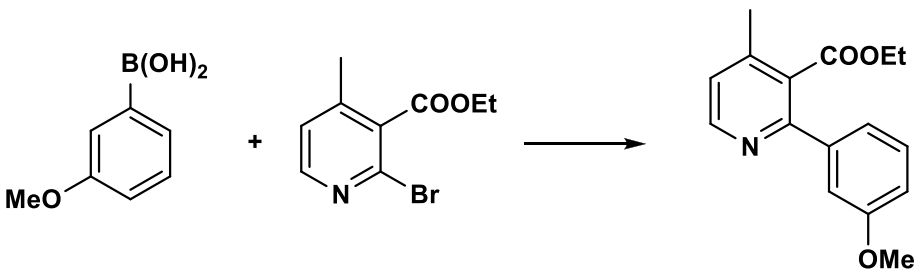


149 **107**

A mixture of ethyl 2-phenyl-4-methylnicotinate **149** (100 mg, 0.68 mmol, 1.0 equiv.) and polyphosphoric acid (9 g, 4.5 mL) was heated at 140 ºC for 5.5 h. The mixture was

poured on crushed ice, neutralised with solid potassium carbonate and partitioned between ethyl acetate (200 mL) and water (60 mL). The aqueous phase was extracted with ethyl acetate (3 × 200 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (25% ethyl acetate-hexane) afforded 1-methyl-4-azafluoren-9-one **107** (62 mg, 78%) as a yellow solid; R_f 0.22 (20% ethyl acetate-hexane); mp 127-129 °C; ν_{max} (solid) 1702 (C=O), 1596, 1564, 1448, 1371, 756 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.35 (d, 1H, J 5.3 Hz, H-3), 7.78 (dd, 1H, J 7.4, 1.2 Hz, H-5), 7.63 (dd, 1H, J 7.4, 1.2 Hz, H-8), 7.52 (td, 1H, J 7.4, 1.2 Hz, H-6), 7.36 (td, 1H, J 7.4, 1.2 Hz, H-7), 6.91 (d, 1H, J 5.3 Hz, H-2), 2.57 (s, 3H, 1-C<u>H</u>$_3$); δ_c (100 MHz, $CDCl_3$) 193.2 (C=O), 165.6 (C-4a), 153.7 (C-3), 147.9 (C-1), 143.9 (C-4b), 135.7 (C-6), 135.5 (C-8a), 131.5 (C-7), 126.6 (C-2), 126.2 (C-9a), 123.9 (C-8), 121.3 (C-5), 16.8 (1-<u>C</u>H_3). *In agreement with published data*.[150]

Ethyl 2-(3-methoxyphenyl)-4-methylnicotinate

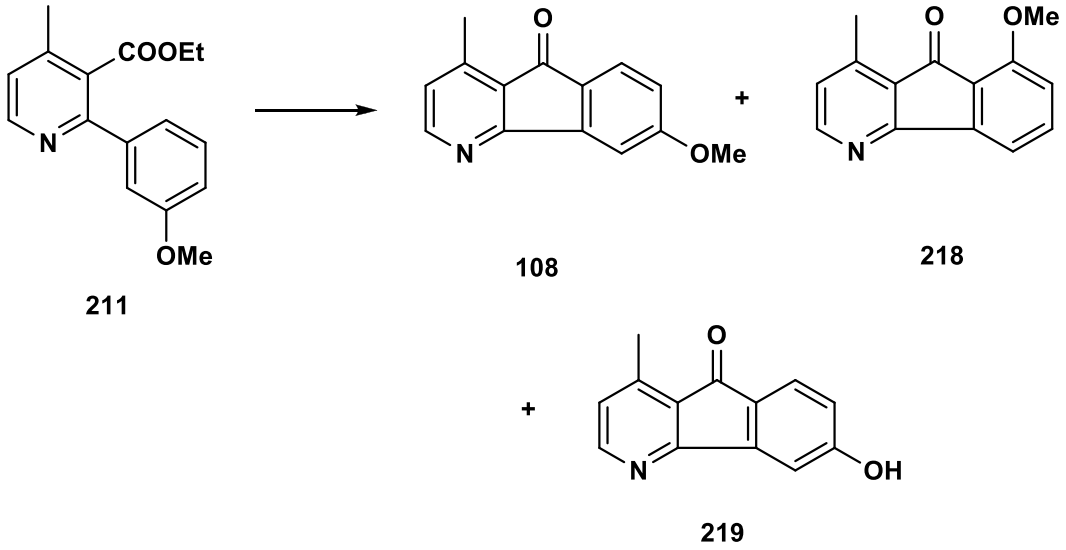


206 **139** **211**

A mixture of **139** (244 mg, 1.00 mmol, 1.0 equiv.), 3-methoxyphenylboronic acid (167 mg, 1.10 mmol, 1.1 equiv.), tetrakis(triphenylphosphine)palladium(0) (116 mg, 0.10 mmol, 0.1 equiv.) and tetrahydrofuran (20 mL) was stirred at 80 ºC for 1 h, then

sodium carbonate solution (2 M aqueous solution; 1.5 mL, 3 mmol, 3.0 equiv.) was added and the resulting mixture stirred at 80 ºC for 18 h. The mixture was diluted with dichloromethane (100 mL) and water (20 mL). The layers were separated and the organic phase washed with water (20 mL) and brine (20 mL). The combined aqueous phases were extracted with dichloromethane (3 × 100 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (14.3% ethyl acetate-hexane) afforded ethyl 2-(3-methoxyphenyl)-4-methylnicotinate **211** (258 mg, 95%) as a colourless oil; R_f 0.19 (16.7% ethyl acetate-hexane); ν_{max} (neat) 2984, 1718 (C=O), 1570, 1464, 1275, 1232, 1118, 1072 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.50 (1H, d, J 5.1 Hz, H-6), 7.25 (1H, t, J 8.0 Hz, H-6'), 7.09-7.06 (3H, m, H-5, H-2', H-5'), 6.88 (1H, ddd, J 8.0, 2.4, 0.8 Hz, H-4'), 4.09 (2H, q, J 7.1 Hz, O-C<u>H</u>$_2$-CH_3), 3.77 (s, 3H, O-C<u>H</u>$_3$), 2.36 (s, 3H, 4-C<u>H</u>$_3$), 0.98 (3H, t, J 7.1 Hz, O-CH_2-C<u>H</u>$_3$); δ_c (100 MHz, $CDCl_3$) 167.6 (C=O), 158.6 (C-3'), 155.4 (C-6), 148.5 (C-2), 144.7 (C-4), 140.2 (C-1'), 128.3 (C-5'), 122.7 (C-3), 122.4 (C-5), 119.7 (C-6'), 114.1 (C-4'), 112.2 (C-2'), 60.5 (O-<u>C</u>H_2-CH_3), 54.3 (O-<u>C</u>H_3), 18.2 (4-<u>C</u>H_3), 12.7 (O-CH_2-<u>C</u>H_3); *m/z* (ESI) 272 $[M+H]^+$ (Found: $[M+H]^+$, 272.1268. $C_{16}H_{17}NO_3$ requires $[M+H]^+$, 272.1287).

1-Methyl-6-methoxy-4-azafluoren-9-one



A mixture of ethyl 2-(3-methoxyphenyl)-4-methylnicotinate **211** (50 mg, 0.18 mmol, 1.0 equiv.) and polyphosphoric acid (3 g, 1.5 mL) was heated at 140 ºC for 5.5 h. The mixture was poured on crushed ice, neutralised with solid potassium carbonate and partitioned between ethyl acetate (80 mL) and water. The aqueous phase was extracted with ethyl acetate (3 × 50 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (25% ethyl acetate-hexane) afforded 1-methyl-6-methoxy-4-azafluoren-9-one **108** (14 mg, 33%) as a yellow solid, 1-methyl-8-methoxy-4-azafluoren-9-one **218** (11 mg, 28%) as a yellow solid and 1-methyl-6-hydroxy-4-azafluoren-9-one **219** (10 mg, 24%) as a yellow solid.

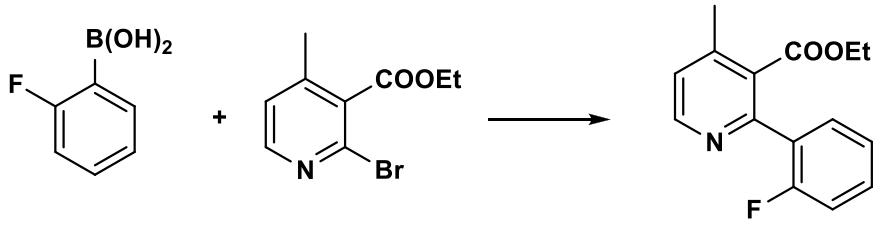
1-Methyl-6-methoxy-4-azafluoren-9-one **108**. R_f 0.41 (40% ethyl acetate-hexane); mp 130-132 °C; ν_{max} (solid) 1695 (C=O), 1610, 1570, 1470, 1435, 1360, 1265, 1225, 1175, 1100, 895, 800 cm^{-1}; 8.35 (d, 1H, J 5.2 Hz, H-3), 7.59 (d, 1H, J 8.2 Hz, H-8), 7.39 (d, 1H, J 2.4 Hz, H-5), 6.97 (d, 1H, J 5.2 Hz, H-2), 6.84 (dd, 1H, J 8.2, 2.4 Hz, H-7), 3.88 (s, 3H, O-C$\underline{H}_3$), 2.59 (s, 3H, Ar-C$\underline{H}_3$); δ_c (100 MHz, $CDCl_3$) 193.7 (C=O), 165.7 (C-4a), 165.3 (C-6), 164.9 (C-3), 164.8 (C-1), 148.5 (C-4b), 127.8 (C-9a), 127.5 (C-8a), 126.6 (C-8), 126.0 (C-2), 120.1 (C-7), 106.8 (C-5), 53.4 (O-$\underline{C}H_3$), 17.4 (1-$\underline{C}H_3$); *m/z* (ESI) 226 $[M+H]^+$ (Found: $[M+H]^+$, 226.0864. $C_{14}H_{11}NO_2$ requires $[M+H]^+$, 226.0868);

HPLC analysis: t_{R1} = 17.327 min, t_{R2} = 21.506 min, t_{R3} = 41.837 min.

1-Methyl-8-methoxy-4-azafluoren-9-one **218**. R_f 0.37 (40% ethyl acetate-hexane); mp 125-127 °C; ν_{max} (solid) 1700 (C=O), 1585, 1565, 1480, 1275, 1150, 1045, 920, 800 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.34 (d, 1H, J 5.2 Hz, H-3), 7.49 (dd, 1H, J 8.4, 7.2 Hz, H-6), 7.43 (dd, 1H, J 7.2, 0.7 Hz, H-5), 6.93-6.82 (m, 2H, H-2, H-7), 3.94 (s, 3H, O-C$\underline{H}_3$), 2.57 (s, 3H, Ar-C$\underline{H}_3$); δ_c (100 MHz, $CDCl_3$) 188.3 (C=O), 166.3 (C-4a), 157.1 (C-8), 152.5 (C-3), 147.3 (C-1), 141.7 (C-4b), 136.4 (C-6), 126.1 (C-9a), 125.3 (C-2), 119.7 (C-8a), 114.1 (C-5), 112.9 (C-7), 55.0 (O-$\underline{C}H_3$), 16.3 (1-$\underline{C}H_3$); *m/z* (ESI) 226 $[M+H]^+$ (Found: $[M+H]^+$, 226.0863. $C_{14}H_{11}NO_2$ requires $[M+H]^+$, 226.0868).

1-Methyl-6-hydroxy-4-azafluoren-9-one **219**. R_f 0.26 (40% ethyl acetate-hexane); mp 240-241 °C; ν_{max} (solid) 2900, 1710 (C=O), 1605, 1570, 1460, 1370, 1320, 1265, 1240, 895, 840 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.36 (d, 1H, J 5.2 Hz, H-3), 7.39 (d, 1H, J 8.4 Hz, H-8), 7.30 (d, 1H, J 2.4 Hz, H-5), 6.91 (d, 1H, J 5.2 Hz, H-2), 6.83 (dd, 1H, J 8.4, 2.4 Hz, H-7), 2.56 (s, 3H, Ar-C$\underline{H}_3$); δ_c (100 MHz, $CDCl_3$) 194.6 (C=O), 170.3 (C-4a), 161.7 (C-6), 155.2 (C-3), 148.5(C-1), 148.1 (C-4b), 137.0 (C-8), 129.6 (C-9a), 129.4 (C-8a), 125.5 (C-2), 116.9 (C-7), 111.9 (C-5), 16.4 (1-$\underline{C}H_3$); *m/z* (ESI) 212 $[M+H]^+$ (Found: $[M+H]^+$, 212.0706. $C_{13}H_9NO_2$ requires $[M+H]^+$, 212.0706); HPLC analysis: t_{R1} = 15.331 min, t_{R2} = 18.610 min, t_{R3} = 33.359 min.

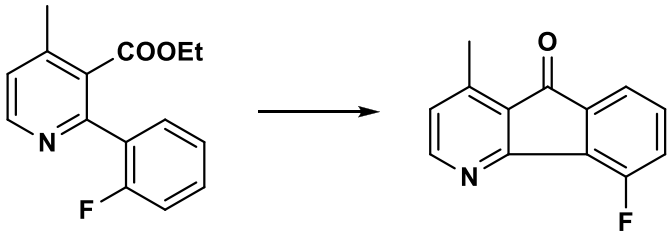
Ethyl 2-(2-fluorophenyl)-4-methylnicotinate



207 **139** **212**

A mixture of **139** (244 mg, 1.00 mmol, 1.0 equiv.), 2-fluorophenylboronic acid (154 mg, 1.10 mmol, 1.1 equiv.), tetrakis(triphenylphosphine)palladium(0) (116 mg, 0.10 mmol, 0.1 equiv.) and tetrahydrofuran (20 mL) was stirred at 80 ºC for 1 h, then sodium carbonate solution (2 M aqueous solution; 1.5 mL, 3 mmol, 3.0 equiv.) was added and the resulting mixture stirred at 80 ºC for 18 h. The mixture was diluted with dichloromethane (100 mL) and water (20 mL). The layers were separated and the organic phase washed with water (20 mL) and brine (20 mL). The combined aqueous phases were extracted with dichloromethane (3 × 100 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (16.7% ethyl acetate-hexane) afforded ethyl 2-(2-fluorophenyl)-4-methylnicotinate **212** (185 mg, 72%) as a colourless oil; R_f 0.28 (25% ethyl acetate-hexane); ν_{max} (neat) 2976, 1721 (C=O), 1582, 1445, 1368, 1239, 1116, 1069, 816 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.54 (1H, d, J 5.0 Hz, H-6), 7.43-7.39 (1H, m, H-6'), 7.13-7.06 (1H, m, H-4'), 7.17-7.13 (2H, m, H-3', H-5), 7.07-7.01 (1H, m, H-5'), 4.06 (2H, q, J 7.1 Hz, O-C<u>H</u>$_2$-CH_3), 2.43 (s, 3H, 4-C<u>H</u>$_3$), 0.94 (3H, t, J 7.1 Hz, O-CH_2-C<u>H</u>$_3$); δ_c (100 MHz, $CDCl_3$) 166.5 (C=O), 158.7 (d, $^1J_{CF}$ 247.7 Hz, C-2'), 151.7 (C-6), 148.9 (C-2), 145.4 (C-4), 130.0 (d, $^3J_{CF}$ 6.8 Hz, C-6'), 129.3 (d, $^3J_{CF}$ 8.1 Hz, C-4'), 128.9 (C-3), 127.0 (d, $^2J_{CF}$ 20.8 Hz, C-1'), 123.6 (C-5), 123.1 (d, $^4J_{CF}$ 3.6 Hz, C-5'), 114.4 (d, $^2J_{CF}$ 21.9 Hz, C-3'), 60.3 (O-<u>C</u>H_2-CH_3), 19.0 (4-<u>C</u>H_3), 12.6 (O-CH_2-<u>C</u>H_3); δ_F (376 MHz, $CDCl_3$) -116.7 (s); *m/z* (ESI) 260 $[M+H]^+$ (Found: $[M+H]^+$, . $C_{15}H_{14}NO_2F$ requires $[M+H]^+$, 260.1087).

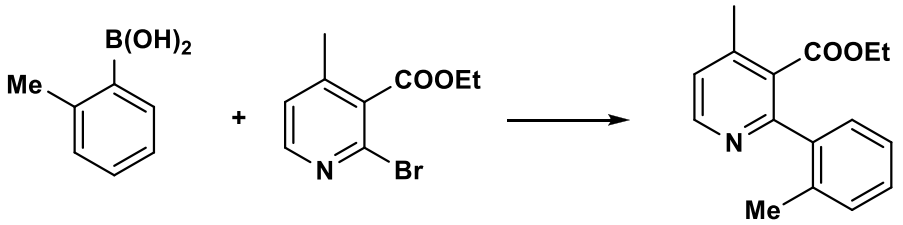
1-Methyl-5-fluoro-4-azafluoren-9-one



212 **110**

A mixture of ethyl 2-(2-fluorophenyl)-4-methylnicotinate **212** (60 mg, 0.23 mmol, 1.0 equiv.) and polyphosphoric acid (4 g, 2 mL) was heated at 140 ºC for 5.5 h. The mixture was poured on crushed ice, neutralised with solid potassium carbonate and partitioned between ethyl acetate (80 mL) and water. The aqueous phase was extracted with ethyl acetate (3 × 50 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (1% dichloromethane-methanol) afforded 1-methyl-5-fluoro-4-azafluoren-9-one **110** (15 mg, 31%) as a yellow solid, with **212** (30 mg, 54%) recovered; R_f 0.19 (10% hexane-acetone); mp 180-181 °C; ν_{max} (solid) 1730 (C=O), 1551, 1523, 1341, 1292, 1224, 826 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.46 (d, 1H, J 5.3 Hz, H-3), 7.47 (dd, 1H, J 7.2, 0.6 Hz, H-8), 7.37-7.32 (m, 1H, H-7), 7.25-7.23 (m, 1H, H-6), 6.94 (d, 1H, J 5.3 Hz, H-2), 2.58 (s, 3H, 1-C<u>H</u>$_3$); δ_c (100 MHz, $CDCl_3$) 191.2 (C=O), 162.8 (C-4a), 156.5 (d, $^1J_{CF}$ 259.6 Hz, C-5), 152.6 (C-3), 147.1 (C-1), 131.9 (d, $^3J_{CF}$ 7.0 Hz, C-7), 127.5 (d, $^3J_{CF}$ 7.0 Hz, C-8a), 124.9 (C-2), 123.4 (C-9a), 122.7 (d, $^2J_{CF}$ 21.1 Hz, C-6), 119.0 (d, $^4J_{CF}$ 3.3 Hz, C-8), 114.6 (d, $^2J_{CF}$ 21.1 Hz, C-4b), 16.6 (1-<u>C</u>H_3); δ_F (376 MHz, $CDCl_3$) -111.1 (s); *m/z* (ESI) 214 $[M+H]^+$ (Found: $[M+H]^+$, 214.0666. $C_{13}H_8NOF$ requires $[M+H]^+$, 214.0668); HPLC analysis: t_{R1} = 17.428 min, t_{R2} = 21.810 min, t_{R3} = 43.698 min.

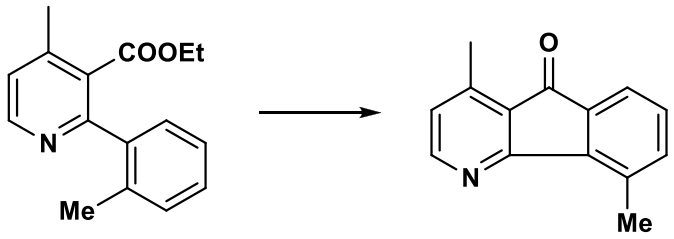
Ethyl 2-(2-methylphenyl)-4-methylnicotinate



208 **139** **213**

A mixture of **139** (244 mg, 1.00 mmol, 1.0 equiv.), 2-methylphenylboronic acid (150 mg, 1.10 mmol, 1.1 equiv.), tetrakis(triphenylphosphine)palladium(0) (116 mg, 0.1 mmol, 0.1 equiv.) and tetrahydrofuran (20 mL) was stirred at 80 ºC for 1 h, then sodium carbonate solution (2 M aqueous solution; 1.5 mL, 3 mmol, 3.0 equiv.) was added and the resulting mixture stirred at 80 ºC for 18 h. The mixture was diluted with dichloromethane (100 mL) and water (20 mL). The layers were separated and the organic phase washed with water (20 mL) and brine (20 mL). The combined aqueous phases were extracted with dichloromethane (3 × 100 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (14.3% ethyl acetate-hexane) afforded ethyl 2-(2-methylphenyl)-4-methylnicotinate **213** (230 mg, 91%) as a colourless oil; R_f 0.34 (16.7% ethyl acetate-hexane); ν_{max} (neat) 2981, 1725 (C=O), 1579, 1442, 1236, 1131, 1061, 812 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.49 (1H, d, J 5.1 Hz, H-6), 7.19-7.14 (2H, m, H-5', H-6'), 7.13-7.06 (3H, m, H-4', H-3', H-5), 3.92 (2H, q, J 7.1 Hz, O-C<u>H</u>$_2$-CH$_3$), 2.37 (s, 3H, 2'-C<u>H</u>$_3$), 2.11 (s, 3H, 4-C<u>H</u>$_3$), 0.80 (3H, t, J 7.1 Hz, O-CH$_2$-C<u>H</u>$_3$); δ_c (100 MHz, $CDCl_3$) 167.9 (C=O), 157.9 (C-6), 149.3 (C-2), 145.4 (C-4), 139.5 (C-2'), 136.2 (C-1'), 130.2 (C-3'), 130.1 (C-4'), 128.5 (C-5'), 128.3 (C-3), 125.2 (C-5), 123.7 (C-6'), 61.1 (O-<u>C</u>H$_2$-CH$_3$), 19.6 (4-<u>C</u>H$_3$), 19.5 (2'-<u>C</u>H$_3$), 13.4 (O-CH$_2$-<u>C</u>H$_3$); *m/z* (ESI) 256 $[M+H]^+$ (Found: $[M+H]^+$, 256.1317. $C_{16}H_{17}NO_2$ requires $[M+H]^+$, 256.1338).

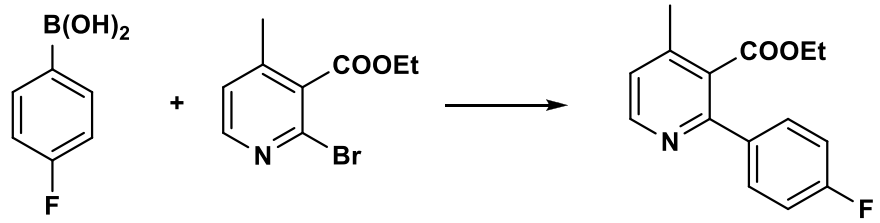
1-Methyl-5-methyl-4-azafluoren-9-one



213 **111**

A mixture of ethyl 2-(2-methylphenyl)-4-methylnicotinate **213** (60 mg, 0.24 mmol, 1.0 equiv.) and polyphosphoric acid (4 g, 2 mL) was heated at 140 ºC for 5.5 h. The mixture was poured on crushed ice, neutralised with solid potassium carbonate and partitioned between ethyl acetate (80 mL) and water. The aqueous phase was extracted with ethyl acetate (3 × 50 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (11.1% ethyl acetate-hexane) afforded 1-methyl-5-methyl-4-azafluoren-9-one **111** (41 mg, 80%) as a yellow solid; R_f 0.61 (16.7% ethyl acetate-hexane); ν_{max} (solid) 2917, 1707 (C=O), 1595, 1560, 1377, 1272, 1224, 948, 829, 759 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.36 (d, 1H, J 5.3 Hz, H-3), 7.46 (dd, 1H, J 7.1, 0.6 Hz, H-8), 7.26 (dd, 1H, J 7.6, 0.6 Hz, H-6), 7.21 (d, 1H, J 7.3 Hz, H-7), 6.84 (d, 1H, J 5.3 Hz, H-2), 2.73 (s, 3H, 1-C$\underline{H}_3$), 2.56 (s, 3H, O-C$\underline{H}_3$); δ_c (100 MHz, $CDCl_3$) 192.7 (C-9), 166.2 (C-4a), 151.3 (C-3), 146.1 (C-1), 138.8 (C-4b), 136.6 (C-5), 134.5 (C-8a), 134.3 (C-6), 129.1 (C-9a), 125.1 (C-7), 123.9 (C-2), 120.2 (C-8), 18.0 (1-$\underline{C}H_3$), 16.4 (5-$\underline{C}H_3$); *m/z* (ESI) 210 $[M+H]^+$ (Found: $[M+H]^+$, 210.0913. $C_{14}H_{11}NO$ requires $[M+H]^+$, 210.0913).

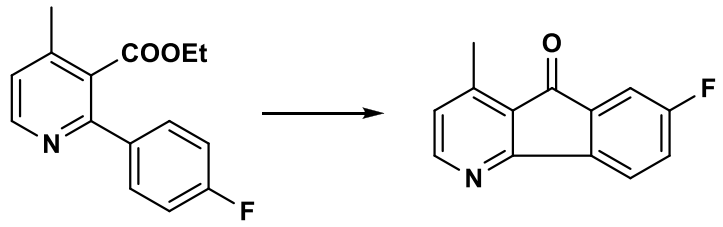
Ethyl 2-(4-fluorophenyl)-4-methylnicotinate



209 139 214

A mixture of **139** (244 mg, 1 mmol, 1.0 equiv.), 4-fluorophenylboronic acid (154 mg, 1.1 mmol, 1.1 equiv.), tetrakis(triphenylphosphine)palladium(0) (116 mg, 0.1 mmol, 0.1 equiv.) and tetrahydrofuran (20 mL) was stirred at 80 ºC for 1 h, then sodium carbonate solution (2 M aqueous solution; 1.5 mL, 3 mmol, 3.0 equiv.) was added and the resulting mixture stirred at 80 ºC for 18 h. The mixture was diluted with dichloromethane (100 mL) and water (20 mL). The layers were separated and the organic phase washed with water (20 mL) and brine (20 mL). The combined aqueous phases were extracted with dichloromethane (3 × 100 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (16.7% ethyl acetate-hexane) afforded ethyl 2-(4-fluorophenyl)-4-methylnicotinate **214** (201 mg, 78%) as a colourless oil; R_f 0.33 (20% ethyl acetate-hexane); ν_{max} (neat) 2985, 1710 (C=O), 1581, 1486, 1375, 1250, 1172, 1083, 837 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.48 (1H, d, J 5.1 Hz, H-6), 7.51 (dd, 2H, J 8.8, 5.4 Hz, H-2', H-6'), 7.10-7.00 (3H, m, H-3', H-5', H-5), 4.09 (2H, q, J 7.1 Hz, O-C<u>H</u>$_2$-CH_3), 2.35 (s, 3H, 4-C<u>H</u>$_3$), 1.00 (3H, t, J 7.1 Hz, O-CH_2-C<u>H</u>$_3$); δ_c (100 MHz, $CDCl_3$) 167.6 (C=O), 162.1 (d, $^1J_{CF}$ 248.2 Hz, C-4'), 154.5 (C-6), 148.6 (C-2), 144.7 (C-4), 135.1 (d, $^4J_{CF}$ 3.3 Hz, C-1'), 129.2 (d, $^3J_{CF}$ 8.4 Hz, C-2', C-6'), 128.2 (C-3), 122.7(C-5), 114.3 (d, $^2J_{CF}$ 21.6 Hz, C-3', C-5'), 60.5 (O-<u>C</u>H_2-CH_3), 18.4 (4-<u>C</u>H_3), 12.7 (O-CH_2-<u>C</u>H_3); δ_F (376 MHz, $CDCl_3$) -113.1 (s); *m/z* (ESI) 260 $[M+H]^+$ (Found: $[M+H]^+$, 260.1080. $C_{15}H_{14}NO_2F$ requires $[M+H]^+$, 260.1087).

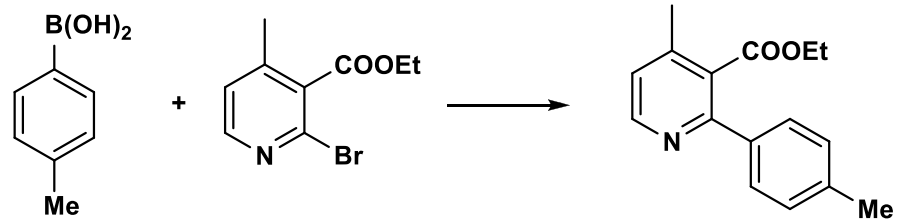
1-Methyl-7-fluoro-4-azafluoren-9-one



214 **112**

A mixture of ethyl 2-(4-fluorophenyl)-4-methylnicotinate **214** (60 mg, 0.23 mmol, 1.0 equiv.) and polyphosphoric acid (4 g, 2 mL) was heated at 140 ºC for 5.5 h. The mixture was poured on crushed ice, neutralised with solid potassium carbonate and partitioned between ethyl acetate (80 mL) and water. The aqueous phase was extracted with ethyl acetate (3 × 50 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (33.3% ethyl acetate-hexane) afforded 1-methyl-7-fluoro-4-azafluoren-9-one **112** (13 mg, 26%) as a yellow solid, with **214** (35 mg, 58%) recovered; R_f 0.54 (1.2% methanol-dichloromethane); mp 160-162 °C; ν_{max} (solid) 1725 (C=O), 1610, 1583, 1310, 1264, 1233, 875 cm^{-1}; δ_H (400 MHz, $CDCl_3$) δ_H (400 MHz, $CDCl_3$) 8.34 (d, 1H, J 5.3 Hz, H-3), 7.74 (dd, 1H, J 7.6, 4.6 Hz, H-5), 7.31 (dd, 1H, J 7.6, 2.4 Hz, H-8), 7.21 (td, 1H, J 7.6, 2.4 Hz, H-6), 6.90 (d, 1H, J 5.3 Hz, H-2), 2.56 (s, 3H, 1-C$\underline{H}_3$); δ_c (100 MHz, $CDCl_3$) 190.7 (C-9), 163.7 (d, $^1J_{CF}$ 252.3 Hz, C-7), 163.6 (C-4a), 151.9 (C-3), 147.0 (C-1), 137.8 (C-9a), 136.2 (d, $^3J_{CF}$ 7.6 Hz, C-8a), 125.2 (d, $^4J_{CF}$ 2.5 Hz, C-4b), 124.6 (C-2), 121.6 (d, $^3J_{CF}$ 7.6 Hz, C-5), 120.4 (d, $^2J_{CF}$ 23.4 Hz, C-6), 110.4 (d, $^2J_{CF}$ 23.9 Hz, C-8), 16.4 (1-$\underline{C}H_3$); δ_F (376 MHz, $CDCl_3$) -108.5 (s); *m/z* (ESI+) 214 $[M+H]^+$ (Found: $[M+H]^+$, 214.0656. $C_{13}H_8NOF$ requires $[M+H]^+$, 214.0668).

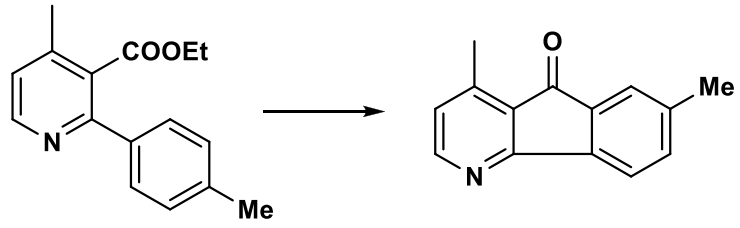
Ethyl 2-(4-methylphenyl)-4-methylnicotinate



210 **139** **215**

A mixture of **139** (244 mg, 1 mmol, 1.0 equiv.), 4-methylphenylboronic acid (150 mg, 1.1 mmol, 1.1 equiv.), tetrakis(triphenylphosphine)palladium(0) (116 mg, 0.1 mmol, 0.1 equiv.) and tetrahydrofuran (20 mL) was stirred at 80 ºC for 1 h, then sodium carbonate solution (2 M aqueous solution; 1.5 mL, 3 mmol, 3.0 equiv.) was added and the resulting mixture stirred at 80 ºC for 18 h. The mixture was diluted with dichloromethane (100 mL) and water (20 mL). The layers were separated and the organic phase washed with water (20 mL) and brine (20 mL). The combined aqueous phases were extracted with dichloromethane (3 × 100 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (16.7% ethyl acetate-hexane) afforded ethyl 2-(4-methylphenyl)-4-methylnicotinate **215** (225 mg, 89%) as a colourless oil; R_f 0.35 (20% ethyl acetate-hexane); ν_{max} (neat) 2981, 1723 (C=O), 1582, 1446, 1239, 1131, 1066, 826 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.48 (1H, d, J 5.0 Hz, H-6), 7.41 (d, 2H, J 8.1, H-2', H-6'), 7.15 (d, 2H, J 8.1, H-3', H-5'), 7.04 (1H, d, J 5.0 Hz, H-5), 4.09 (2H, q, J 7.1 Hz, O-C<u>H</u>$_2$-CH$_3$), 2.34 (s, 3H, 2'-C<u>H</u>$_3$), 2.31 (s, 3H, 4-C<u>H</u>$_3$),1.00 (3H, t, J 7.1 Hz, O-CH$_2$-C<u>H</u>$_3$); δ_c (100 MHz, $CDCl_3$) 167.2 (C=O), 154.9 (C-6), 147.9 (C-2), 143.8 (C-4), 136.9 (C-1'), 135.5 (C-4'), 127.5 (C-3', C-5'), 127.4 (C-2', C-6'), 126.5 (C-3), 121.6 (C-5), 59.8 (O-<u>C</u>H$_2$-CH$_3$), 19.6 (4'-<u>C</u>H$_3$), 17.8 (4-<u>C</u>H$_3$), 12.1 (O-CH$_2$-<u>C</u>H$_3$); *m/z* (ESI) 256 $[M+H]^+$ (Found: $[M+H]^+$, 256.1327. $C_{16}H_{17}NO_2$ requires $[M+H]^+$, 256.1338).

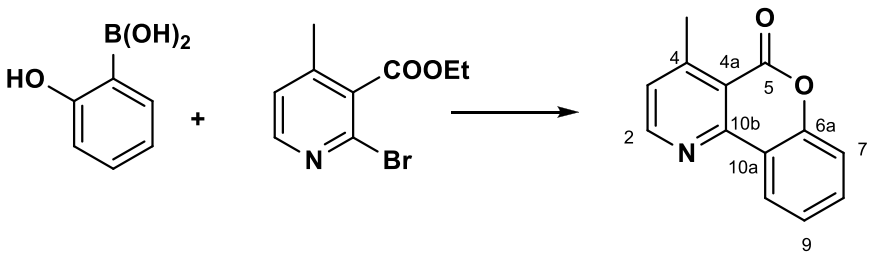
1-Methyl-7-methyl-4-azafluoren-9-one



215 **113**

A mixture of ethyl 2-(4-methylphenyl)-4-methylnicotinate **215** (60 mg, 0.24 mmol, 1.0 equiv.) and polyphosphoric acid (4 g, 2 mL) was heated at 140 ºC for 5.5 h. The mixture was poured on crushed ice, neutralised with solid potassium carbonate and partitioned between ethyl acetate (80 mL) and water. The aqueous phase was extracted with ethyl acetate (3 × 50 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (20% ethyl acetate-hexane) afforded 1-methyl-7-methyl-4-azafluoren-9-one **113** (37 mg, 75%) as a yellow solid; R_f 0.31 (20% ethyl acetate-hexane); mp 151-153 °C; ν_{max} (solid) 1715 (C=O), 1599, 1568, 1379, 1280, 1246, 830 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.31 (d, 1H, J 5.3 Hz, H-3), 7.65 (d, 1H, J 7.6 Hz, H-5), 7.43 (d, 1H, J 0.8 Hz, H-8), 7.31 (dd, 1H, J 7.6, 0.8 Hz, H-6), 6.86 (d, 1H, J 5.3 Hz, H-2), 2.55 (s, 3H, 1-C$\underline{H}_3$), 2.35 (s, 3H, 7-C$\underline{H}_3$); δ_c (100 MHz, $CDCl_3$) 192.2 (C-9), 164.1 (C-4a), 151.3 (C-3), 146.1 (C-1), 140.0 (C-4b), 139.1 (C-7), 134.2 (C-8a), 133.9 (C-6), 124.7 (C-8), 124.2 (C-9a), 123.0 (C-5), 119.3 (C-2), 20.3 (7-$\underline{C}H_3$), 16.0 (1-$\underline{C}H_3$); *m/z* (ESI) 210 $[M+H]^+$ (Found: $[M+H]^+$, 210.0909. $C_{14}H_{11}NO$ requires $[M+H]^+$, 210.0913).

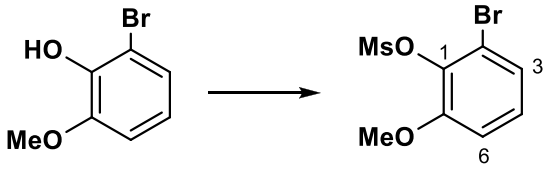
4-Methylpyrido[3,2-*c*]coumarin



155 **139** **159**

A mixture of **139** (100 mg, 0.41 mmol, 1.0 equiv.), 2-hydroxyphenylboronic acid (62 mg, 0.45 mmol, 1.1 equiv.), tetrakis(triphenylphosphine)palladium(0) (47 mg, 0.04 mmol, 0.1 equiv.) and tetrahydrofuran (20 mL) was stirred at 80 ºC for 1 h, then sodium carbonate solution (2 M aqueous solution; 0.62 mL, 1.23 mmol, 3.0 equiv.) was added and the resulting mixture stirred at 80 ºC for 18 h. The mixture was diluted with dichloromethane (100 mL) and water (20 mL). The layers were separated and the organic phase washed with water (20 mL) and brine (20 mL). The combined aqueous phases were extracted with dichloromethane (3 × 100 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (6.7% ethyl acetate-hexane) afforded 4-methylpyrido[3,2-*c*]coumarin **159** (82 mg, 92%) as a white solid. R_f 0.58 (25% ethyl acetate-hexane); mp 156-158 °C; ν_{max} (solid) 2957, 1731 (C=O), 1580, 1506, 1472, 762 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.74 (d, 1H, J 4.9 Hz, H-2), 8.53 (dd, 1H, J 7.4, 1.2 Hz, H-10), 7.50 (td, 1H, J 7.4, 1.2 Hz, H-8), 7.33-7.27 (m, 2H, H-7, H-9), 7.24 (d, 1H, J 4.9 Hz, H-3), 2.84 (s, 3H, 4-C<u>H</u>$_3$); δ_c (100 MHz, $CDCl_3$) 159.5 (C=O), 153.2 (C-2), 152.5 (C-6a), 152.0 (C-10b), 151.4 (C-4), 131.1 (C-10a), 125.7 (C-8a), 124.3 (C-10), 123.6 (C-8), 118.5 (C-9), 115.7 (C-3), 115.5 (C-7), 22.0 (4-<u>C</u>H_3); *m/z* (ESI) 212 $[M+H]^+$ (Found: $[M+H]^+$, 212.0704. $C_{13}H_9NO_2$ requires $[M+H]^+$, 212.0712). *In agreement with published data*.[132]

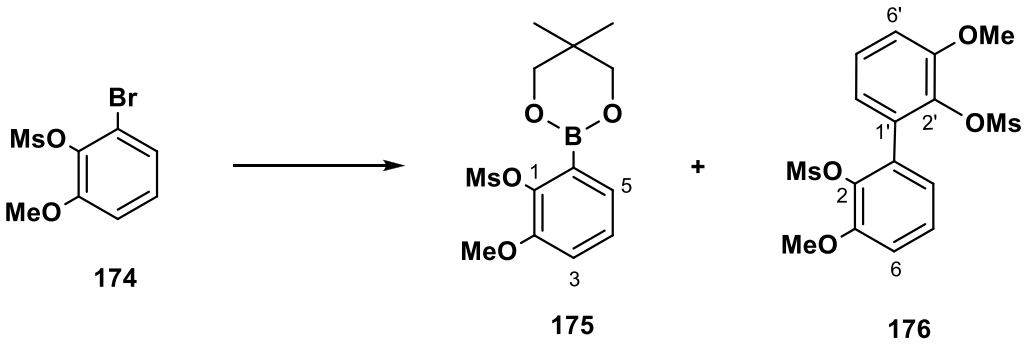
2-Bromo-6-methoxyphenyl methanesulfonate



173 **174**

To a solution of 2-bromo-6-methoxyphenol **173** (1.50 g, 7.39 mmol, 1.0 equiv.) in dichloromethane (20 mL) was added triethylamine (2.05 mL, 14.78 mmol, 2.0 equiv.) and methanesulfonyl chloride (694 µL, 8.87 mmol, 1.2 equiv.) at 0 ºC. The mixture was stirred for 6 h at rt. The mixture was partitioned between dichloromethane (200 mL) and water (50 mL). The aqueous phase was extracted with dichloromethane (3 × 200 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (20% ethyl acetate-hexane) afforded 2-bromo-6-methoxyphenyl methanesulfonate **174** (1.84 g, 89%) as a white solid; R_f 0.27 (25% ethyl acetate-hexane); mp 71-72 °C; ν_{max} (solid) 2920, 1469, 1366, 1275, 1252, 1161, 1029, 965, 800 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.14 (1H, dd, J 8.2, 1.2 Hz, H-3), 7.05 (1H, t, J 8.2 Hz, H-4), 6.88 (1H, dd, J 8.4, 1.2 Hz, H-5), 3.84 (s, 3H, O-C$\underline{H}_3$), 3.33 (s, 3H, SO_2-C$\underline{H}_3$); δ_C (100 MHz, $CDCl_3$) 153.8 (C-6), 139.5 (C-1), 131.3 (C-3), 123.7 (C-4), 121.5 (C-2), 113.3 (C-5), 56.8 (O-$\underline{C}H_3$), 40.7 (SO_2 -$\underline{C}H_3$); *m/z* (ESI) 281 $[M+H]^+$ (Found: $[M+H]^+$, 280.9471. $C_8H_9O_4SBr$ requires $[M+H]^+$, 280.9483).

2-Methoxy-6-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)phenyl methanesulfonate



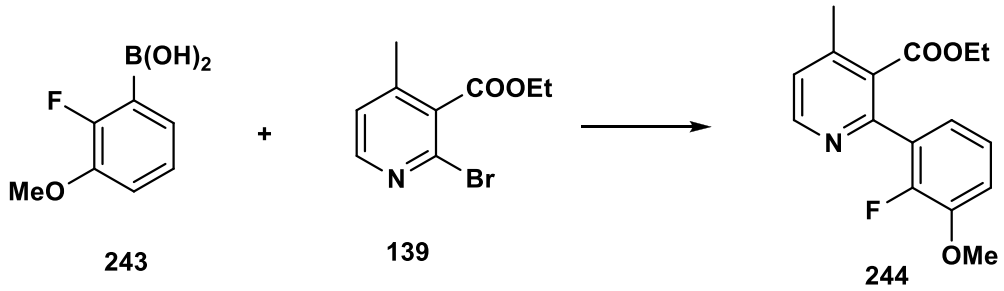
A mixture of 2-bromo-6-methoxyphenyl methanesulfonate **174** (140 mg, 0.50 mmol, 1.0 equiv.), bis(neopentyl glycolato)diboron (136 mg, 0.60 mmol, 1.2 equiv.), potassium acetate (147 mg, 1.50 mmol, 3.0 equiv.), [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (20 mg, 0.025 mmol, 0.05 equiv.) and dimethyl sulfoxide (5.0 mL) was stirred at 80 ºC for 12 h. The reaction solution was cooled to rt and poured into ice water. The mixture was partitioned between ethyl acetate (200 mL) and water (40 mL). The aqueous phase was extracted with ethyl acetate (3 × 200 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (dichloromethane: methanol: triethylamine = 80:2:1) gave 2-methoxy-6-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)phenyl methanesulfonate **175** (11 mg, 7%) as a colourless oil and 2,2'-methanesulfonyloxy-3,3'-dimethoxybiphenyl **176** (72 mg, 36%) as a colourless oil.

2-Methoxy-6-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)phenyl methanesulfonate **175**. R_f 0.26 (dichloromethane: methanol: triethylamine = 80:2:1); ν_{max} (neat) 2891, 1561, 1408, 1364, 1225, 1162, 1042, 902, 793 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.25 (1H, dd, J 7.6, 1.6 Hz, H-5), 7.16 (1H, t, J 7.6 Hz, H-4), 6.98 (1H, dd, J 7.6, 1.6 Hz, H-3), 3.81 (s, 3H, O-C$\underline{H}_3$), 3.72 (4H, s, O-C$\underline{H}_2$-), 3.11 (s, 3H, SO_2-C$\underline{H}_3$), 0.98 (6H, s, C-(C$\underline{H}_3$)$_2$); δ_C (100 MHz, $CDCl_3$) 150.2 (C-6), 141.2 (C-1), 126.4 (C-3), 126.0 (C-4), 113.9 (C-5), 112.7 (C-2), 71.3 (O-$\underline{C}H_2$-), 55.1 (O-$\underline{C}H_3$), 37.9 (SO_2 -$\underline{C}H_3$), 30.8 ($\underline{C}$-$(CH_3)_2$), 30.0

(C-($\underline{C}H_3$)$_2$); *m/z* (ESI) 315 $[M+H]^+$ (Found: $[M+H]^+$, 315.1082. $C_{13}H_{19}O_6SB$ requires $[M+H]^+$, 315.1074).

2,2'-Methanesulfonyloxy-3,3'-dimethoxybiphenyl **176**. R_f 0.72 (dichloromethane: methanol: triethylamine = 80:2:1); ν_{max} (neat) 2870, 1621, 1534, 1368, 1273, 1246, 1168, 1035, 954, 785 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.25 (2H, t, J 8.4 Hz, H-5, H-5'), 7.00-6.96 (4H, m, H-4, H-4', H-6, H-6'), 3.88 (6H, s, O-C$\underline{H}_3$ × 2), 2.70 (6H, s, SO_2-C$\underline{H}_3$ × 2); δ_C (100 MHz, $CDCl_3$) 151.5 (C-3, C-3'), 135.7 (C-2, C-2'), 130.4 (C-1, C-1'), 126.1 (C-6, C-6'), 122.2 (C-5, C-5'), 111.4 (C-4, C-4'), 55.2 (O-$\underline{C}H_3$), 38.1 (SO_2 -$\underline{C}H_3$); *m/z* (ESI) 403 $[M+H]^+$ (Found: $[M+H]^+$, 403.0529. $C_{16}H_{18}O_8S_2$ requires $[M+H]^+$, 403.0521).

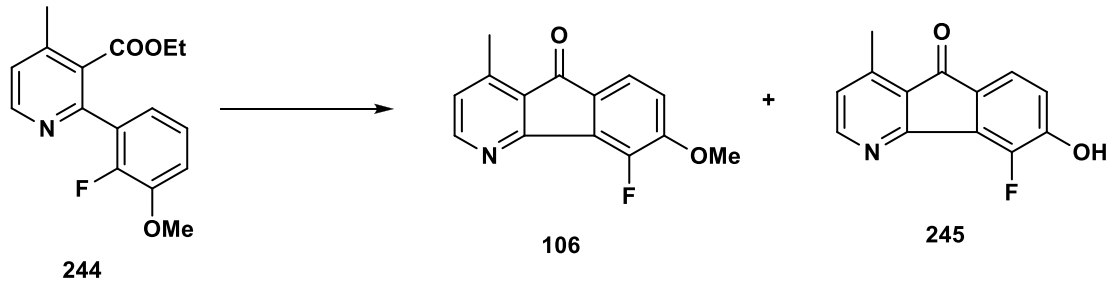
Ethyl 2-(2-fluoro-3-methoxyphenyl)-4-methylnicotinate



To a mixture of 2-fluoro-3-methoxyphenylboronic acid **243** (2.50 g, 14.71 mmol, 1.0 equiv.), **139** (3.95 g, 16.18 mmol, 1.1 equiv.), tetrakis(triphenylphosphine)palladium(0) (1.87 g, 1.618 mmol, 0.11 equiv.) was added tetrahydrofuran (80 mL). The mixture was stirred at 80 ºC for 1 h, then sodium carbonate solution (2 M aqueous solution; 18.4 mL, 36.8 mmol, 2.5 equiv.) was added and the resulting mixture stirred at 80 ºC for 20 h. The reaction solution was cooled to rt and diluted with dichloromethane (1 L) and water (100 mL). The layers were separated and the organic phase washed with brine (50 mL).

The combined aqueous phases were extracted with dichloromethane (3 × 500 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (33.3% ethyl acetate-hexane) afforded ethyl 2-(2-fluoro-3-methoxyphenyl)-4-methylnicotinate **244** (3.99 g, 94%) as a colourless oil; R_f 0.33 (25% ethyl acetate-hexane); ν_{max} (neat) 2970, 1721 (C=O), 1442, 1366, 1268, 1217, 1121, 1054, 785 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.58 (1H, d, J 5.1 Hz, H-6), 7.19 (1H, d, J 5.1 Hz, H-5), 7.12 (1H, td, J 8.1, 1.2 Hz, H-6'), 7.03-6.98 (2H, m, H-4', H-5'), 4.14 (2H, q, J 7.1 Hz, O-C<u>H</u>$_2$-CH_3), 3.89 (s, 3H, O-C<u>H</u>$_3$), 2.47 (s, 3H, 1-C<u>H</u>$_3$), 1.03 (3H, t, J 7.1 Hz, O-CH_2-C<u>H</u>$_3$); δ_c (100 MHz, $CDCl_3$) 167.5 (C=O), 152.5 (C-6), 149.9 (C-2), 149.8 (d, $^1J_{CF}$ 246.4 Hz, C-2'), 147.8 (d, $^2J_{CF}$ 10.9 Hz, C-3'), 146.5 (C-4), 130.1 (C-3), 128.8 (d, $^2J_{CF}$ 12.7 Hz, C-1'), 124.8 (C-5), 124.0 (d, $^3J_{CF}$ 4.7 Hz, C-6'), 122.3 (d, $^4J_{CF}$ 2.0 Hz, C-5'), 129.3 (d, $^3J_{CF}$ 1.9 Hz, C-4'), 61.4 (O-<u>C</u>H_2-CH_3), 56.6 (O-<u>C</u>H_3), 20.2 (4-<u>C</u>H_3), 13.7 (O-CH_2-<u>C</u>H_3); δ_F (376 MHz, $CDCl_3$) -139.0 (s); *m/z* (ESI) 290 $[M+H]^+$ (Found: $[M+H]^+$, 290.1188. $C_{16}H_{16}NO_3F$ requires $[M+H]^+$, 290.1193).

1-Methyl-5-fluoro-6-methoxy-4-azafluoren-9-one



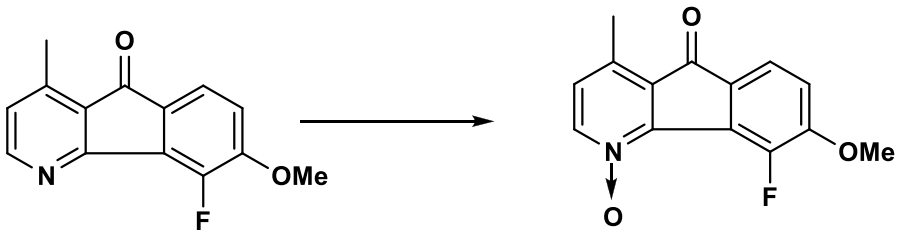
A mixture of ethyl 2-(2-fluoro-3-methoxyphenyl)-4-methylnicotinate **244** (2.07 g, 7.16 mmol, 1.0 equiv.) and polyphosphoric acid (36.8 g, 17.9 mL) was stirred at 130 ºC for 10.5 h. The mixture was poured on crushed ice, neutralised with saturated potassium hydroxide solution and partitioned between chloroform (1 L) and water. The aqueous phase was extracted with chloroform (3 × 1 L) and the combined organic phases dried

(Na_2SO_4). Concentration under reduced pressure and chromatography (1% methanol-dichloromethane) afforded 1-methyl-5-fluoro-6-methoxy-4-azafluoren-9-one **106** (930 mg, 53%) as a yellow solid and 1-methyl-5-fluoro-6-hydroxy-4-azafluoren-9-one **245** (219 mg, 13%) as a yellow solid.

1-Methyl-5-fluoro-6-methoxy-4-azafluoren-9-one **106.** R_f 0.25 (1% methanol-dichloromethane); mp 189-190 °C; ν_{max} (solid) 3022, 1699 (C=O), 1569, 1496, 1271, 1238, 1084, 992, 796, 776 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.52 (d, 1H, J 5.3 Hz, H-3), 7.50 (d, 1H, J 8.1 Hz, H-8), 7.00 (d, 1H, J 5.3 Hz, H-2), 6.97 (dd, 1H, J 7.9, 7.2 Hz, H-7), 3.98 (s, 3H, O-C$\underline{H}_3$), 2.64 (s, 3H, 1-C$\underline{H}_3$); δ_c (100 MHz, $CDCl_3$) 191.1 (C=O), 162.8 (C-4a), 154.7 (d, $^2J_{CF}$ 10.2 Hz, C-6), 153.0 (C-3), 147.8 (C-1), 147.1 (d, $^1J_{CF}$ 260.4 Hz, C-5), 132.2 (d, $^2J_{CF}$ 10.0 Hz, C-4b), 129.0 (d, $^3J_{CF}$ 9.0 Hz, C-8a), 128.7 (d, $^3J_{CF}$ 12.2 Hz, C-7), 127.1 (C-9a), 126.1 (C-2), 121.1 (d, $^4J_{CF}$ 3.5 Hz, C-8), 56.9 (O-$\underline{C}H_3$), 17.5 (1-$\underline{C}H_3$); δ_F (376 MHz, $CDCl_3$) -137.7 (s); *m/z* (ESI) 244 $[M+H]^+$ (Found: $[M+H]^+$, 244.0766. $C_{14}H_{10}NO_2F$ requires $[M+H]^+$, 244.0768); HPLC analysis: t_{R1} = 16.688 min, t_{R2} = 20.580 min, t_{R3} = 39.533 min.

1-Methyl-5-fluoro-6-hydroxy-4-azafluoren-9-one **245**. R_f 0.07 (1% methanol-dichloromethane); mp 237-238 °C; ν_{max} (solid) 3080, 1712 (C=O), 1574, 1511, 1277, 1263, 1238, 990, 818, 797, 770, 668 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.43 (d, 1H, J 5.3 Hz, H-3), 7.38 (d, 1H, J 8.0 Hz, H-8), 7.00 (d, 1H, J 5.3 Hz, H-2), 6.96 (t, 1H, J 7.6 Hz, H-7), 2.66 (s, 3H, 1-C$\underline{H}_3$); δ_c (100 MHz, $CDCl_3$) 190.8 (C=O), 162.1 (C-4a), 152.9 (d, $^2J_{CF}$ 12.8 Hz, C-6), 151.3 (C-3), 148.5 (C-1), 146.1 (d, $^1J_{CF}$ 255.5 Hz, C-5), 128.6 (d, $^2J_{CF}$ 9.7 Hz, C-4b), 127.2 (C-9a), 127.0 (d, $^3J_{CF}$ 4.7 Hz, C-8a), 126.2 (C-2), 121.6 (d, $^4J_{CF}$ 2.8 Hz, C-8), 119.6 (d, $^3J_{CF}$ 2.8 Hz, C-7), 17.1 (1-$\underline{C}H_3$); δ_F (376 MHz, $CDCl_3$) -136.2 (s); *m/z* (ESI) 230 $[M+H]^+$ (Found: $[M+H]^+$, 230.0611. $C_{13}H_8NO_2F$ requires $[M+H]^+$, 230.0617); HPLC analysis: t_{R1} = 14.884 min, t_{R2} = 17.845 min, t_{R3} = 30.685 min.

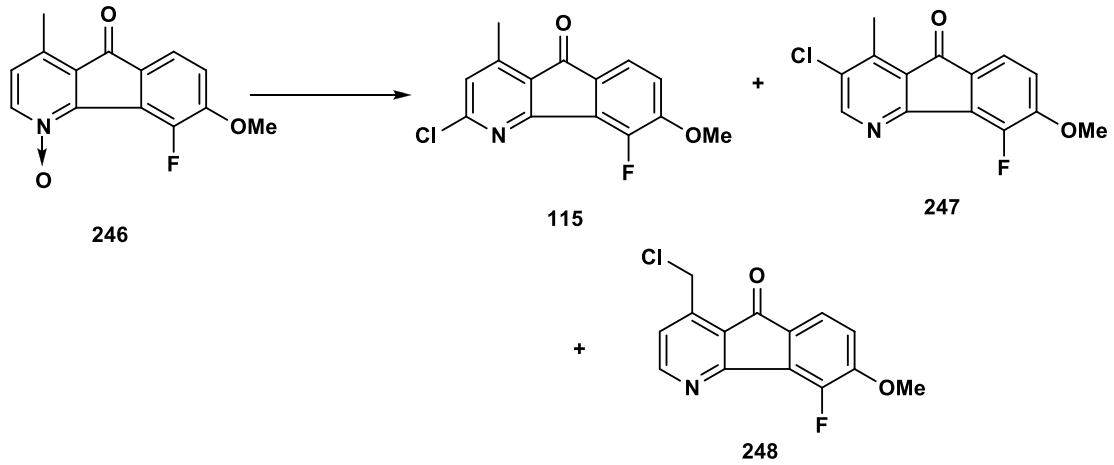
1-Methyl-5-fluoro-6-methoxy-4-azafluoren-9-one *N*-oxide



106 **246**

To a solution of 1-methyl-5-fluoro-6-methoxy-4-azafluoren-9-one **106** (897 mg, 3.68 mmol) in acetic acid (68 mL), 30% H_2O_2 (6.8 mL) was added at rt. The mixture was stirred at 80 ºC for 9 h. Then the reaction solution was cooled to rt and neutralised with sodium carbonate solid in the ice bath. The mixture was partitioned between chloroform (500 mL) and water (40 mL). The aqueous phase was extracted with chloroform (3×500 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (2.5% methanol-dichloromethane) afforded 1-methyl-5-fluoro-6-methoxy-4-azafluoren-9-one *N*-oxide **246** (582 mg, 61%) as an orange solid; R_f 0.31 (50% acetone-hexane); mp 236-238 °C; ν_{max} (solid) 3031, 1708(C=O), 1583, 1442, 1268, 1225, 1076, 898, 787, 769 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.14 (d, 1H, J 4.3 Hz, H-3), 7.52 (d, 1H, J 8.1 Hz, H-8), 7.01 (d, 1H, J 4.4 Hz, H-2), 6.93 (t, 1H, J 7.1 Hz, H-7), 3.93 (s, 3H, O-C$\underline{H}_3$), 2.60 (s, 3H, 1-C$\underline{H}_3$); δ_c (100 MHz, $CDCl_3$ + CD_3OD) 187.7 (C=O), 156.0 (d, $^2J_{CF}$ 12.2 Hz, C-6), 149.4 (C-4a), 146.7 (d, $^1J_{CF}$ 270.1 Hz, C-5), 143.9 (C-3), 139.2 (C-1), 129.3 (C-2), 127.6 (C-9a), 124.2 (d, $^3J_{CF}$ 3.3 Hz, C-8a), 121.9 (d, $^3J_{CF}$ 12.2 Hz, C-7), 119.4 (d, $^2J_{CF}$ 13.6 Hz, C-4b), 114.3 (d, $^4J_{CF}$ 1.5 Hz, C-8), 56.7 (O-$\underline{C}H_3$), 16.3 (1-$\underline{C}H_3$); δ_F (376 MHz, $CDCl_3$) -133.3 (s);

1-Methyl-3-chloro-5-fluoro-6-methoxy-4-azafluoren-9-one



A mixture of 1-methyl-5-fluoro-6-methoxy-4-azafluoren-9-one *N*-oxide **246** (141 mg, 0.55 mmol) and phosphoric trichloride (11.0 mL) was stirred at 100 ºC for 1 h. Then the reaction solution was cooled to rt. Ice water was added and the mixture neutralised with sodium carbonate solid. The resulting mixture was partitioned between dichloromethane (500 mL) and water. The aqueous phase was extracted with dichloromethane (3 × 250 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (33.3% dichloromethane-hexane) afforded 1-methyl-3-chloro-5-fluoro-6-methoxy-4-azafluoren-9-one **115** (51 mg, 34%) as a yellow solid, 1-methyl-2-chloro-5-fluoro-6-methoxy-4-azafluoren-9-one **247** (26 mg, 17%) as a yellow solid and 1-chloromethyl-5-fluoro-6-methoxy-4-azafluoren-9-one **248** (28 mg, 18%) as a yellow solid.

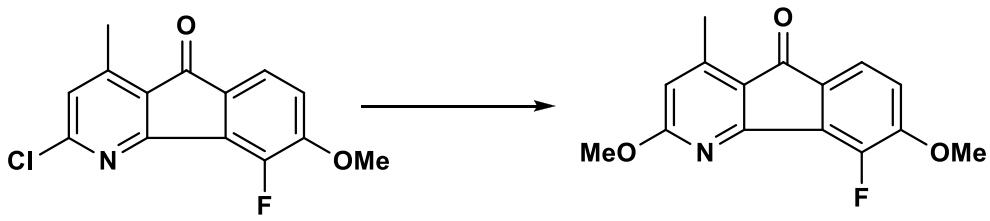
1-Methyl-3-chloro-5-fluoro-6-methoxy-4-azafluoren-9-one **115**. R_f 0.43 (dichloromethane); mp 202-204 °C; ν_{max} (solid) 2926, 1710 (C=O), 1561, 1500, 1372, 1281, 1240, 898, 791 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.50 (d, 1H, J 8.1 Hz, H-8), 7.06 (s, 1H, H-2), 6.93 (dd, 1H, J 8.0, 7.0 Hz, H-7), 3.99 (s, 3H, O-C$\underline{H}_3$), 2.62 (s, 3H, 1-C$\underline{H}_3$); δ_c (100 MHz, $CDCl_3$) 189.9 (C=O), 163.8 (C-4a), 155.8 (C-3), 154.8 (d, $^2J_{CF}$ 10.0 Hz, C-6), 149.8 (C-1), 147.1 (d, $^1J_{CF}$ 264.1 Hz, C-5), 128.6 (d, $^3J_{CF}$ 4.4 Hz, C-8a), 128.2 (d,

$^{2}J_{CF}$ 22.9 Hz, C-4b), 125.8 (d, $^{3}J_{CF}$ 8.2 Hz, C-7), 125.7 (C-9a), 121.2 (d, $^{4}J_{CF}$ 3.5 Hz, C-8), 114.3 (C-2), 57.0 (O-$\underline{C}H_3$), 17.3 (1-$\underline{C}H_3$); δ_F (376 MHz, $CDCl_3$) -135.7 (s); *m/z* (ESI) 278 $[M+H]^+$ (Found: $[M+H]^+$, 278.0378. $C_{14}H_9NO_2FCl$ requires $[M+H]^+$, 278.0384).

1-Methyl-2-chloro-5-fluoro-6-methoxy-4-azafluoren-9-one **247**. R_f 0.37 (dichloromethane); mp 170-172 °C; ν_{max} (solid) 2938, 1709 (C=O), 1565, 1487, 1374, 1269, 1136, 1061, 789 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.57 (s, 1H, H-3), 7.51 (dd, 1H, J 8.0, 0.4 Hz, H-8), 6.97 (dd, 1H, J 8.0, 7.1 Hz, H-7), 3.99 (s, 3H, O-C$\underline{H}_3$), 2.70 (s, 3H, 1-C$\underline{H}_3$); δ_c (100 MHz, $CDCl_3$) 190.0 (C=O), 160.9 (C-4a), 154.9 (d, $^{2}J_{CF}$ 10.1 Hz, C-6), 152.2 (d, $^{1}J_{CF}$ 227.6 Hz, C-5), 152.1 (C-3), 145.8 (C-1), 133.9 (C-2), 128.4 (d, $^{2}J_{CF}$ 24.5 Hz, C-4b), 127.8 (C-9a), 124.9 (d, $^{3}J_{CF}$ 4.9 Hz, C-7), 124.1 (d, $^{3}J_{CF}$ 4.6 Hz, C-8a), 121.5 (d, $^{4}J_{CF}$ 3.6 Hz, C-8), 56.9 (O-$\underline{C}H_3$), 13.9 (1-$\underline{C}H_3$); δ_F (376 MHz, $CDCl_3$) -137.6 (s); *m/z* (ESI) 278 $[M+H]^+$ (Found: $[M+H]^+$, 278.0376. $C_{14}H_9NO_2FCl$ requires $[M+H]^+$, 278.0384).

1-Chloromethyl-5-fluoro-6-methoxy-4-azafluoren-9-one **248**. R_f 0.30 (dichloromethane); mp 176-178 °C; ν_{max} (solid) 2960, 1707 (C=O), 1568, 1508, 1373, 1264, 1226, 1048, 794, 730 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.64 (d, 1H, J 5.4 Hz, H-3), 7.47 (d, 1H, J 8.1 Hz, H-8), 7.33 (d, 1H, J 5.4 Hz, H-2), 6.88 (dd, 1H, J 7.9, 7.1 Hz, H-7), 4.99 (2H, s, 1-ClC$\underline{H}_2$), 3.99 (s, 3H, O-C$\underline{H}_3$); δ_c (100 MHz, $CDCl_3$) 189.0 (C=O), 161.6 (C-4a), 153.9 (d, $^{2}J_{CF}$ 10.0 Hz, C-6), 153.3 (C-3), 146.0 (d, $^{1}J_{CF}$ 262.7 Hz, C-5), 144.3 (C-1), 128.0 (d, $^{2}J_{CF}$ 9.1 Hz, C-4b), 127.0 (d, $^{3}J_{CF}$ 6.2 Hz, C-8a), 124.4 (C-9a), 122.2 (C-2), 120.5 (d, $^{3}J_{CF}$ 3.6 Hz, C-7), 112.9 (d, $^{4}J_{CF}$ 1.4 Hz, C-8), 55.7 (O-$\underline{C}H_3$), 38.5 (1-$\underline{C}H_2$); δ_F (376 MHz, $CDCl_3$) -137.2 (s); *m/z* (ESI) 278 $[M+H]^+$ (Found: $[M+H]^+$, 278.0378. $C_{14}H_9NO_2FCl$ requires $[M+H]^+$, 278.0384).

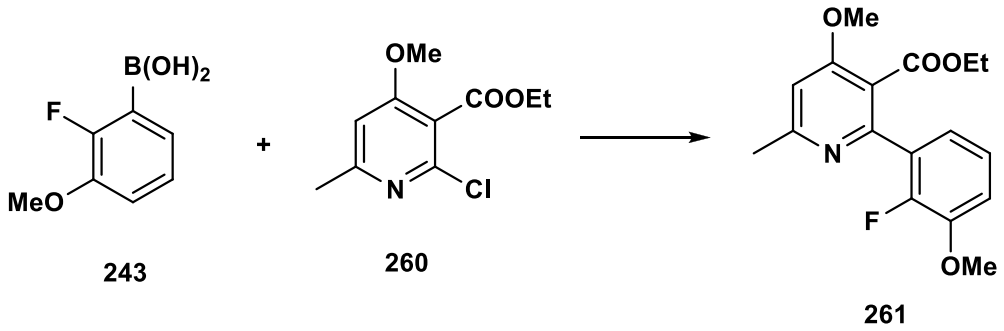
1-Methyl-3-methoxy-5-fluoro-6-methoxy-4-azafluoren-9-one



115 **116**

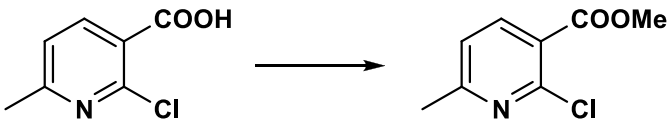
To a mixture of 1-methyl-3-chloro-5-fluoro-6-methoxy-4-azafluoren-9-one **115** (42 mg, 0.15 mmol, 1.0 equiv.), sodium methoxide (123 mg, 2.27 mmol, 15.0 equiv.), anhydrous methanol (5.0 mL) was added at rt. The resulting solution was refluxed for 4 h, then cooled to rt and water (25 mL) was added. The mixture was extracted with dichloromethane (3 × 250 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (14.3% acetone-hexane) afforded 1-methyl-3-methoxy-5-fluoro-6-methoxy-4- azafluoren-9-one **116** (21 mg, 51%) as a yellow solid; R_f 0.30 (14.3% acetone-hexane); mp 220-221 °C; ν_{max} (solid) 2926, 1693 (C=O), 1560, 1500, 1364, 1280, 1066, 790, 778 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.41 (d, 1H, J 8.0 Hz, H-8), 6.95 (dd, 1H, J 8.0, 7.1 Hz, H-7), 6.38 (s, 1H, H-2), 4.01 (s, 3H, 6-O-C$\underline{H}_3$), 3.96 (s, 3H, 3-O-C$\underline{H}_3$), 2.55 (s, 3H, 1-C$\underline{H}_3$); δ_c (100 MHz, $CDCl_3$) 190.6 (C=O), 168.1 (C-3), 163.8 (C-4a), 154.1 (d, $^2J_{CF}$ 10.1 Hz, C-6), 149.8 (C-1), 146.8 (d, $^1J_{CF}$ 262.0 Hz, C-5), 129.3 (d, $^3J_{CF}$ 7.1 Hz, C-8a), 128.4 (d, $^2J_{CF}$ 9.0 Hz, C-4b), 121.4 (C-9a), 120.2 (d, $^4J_{CF}$ 3.5 Hz, C-8), 113.2 (d, $^3J_{CF}$ 4.2 Hz, C-7), 111.0 (C-2), 56.8 (6-O-$\underline{C}H_3$), 54.4 (3-O-$\underline{C}H_3$), 17.8 (1-$\underline{C}H_3$); δ_F (376 MHz, $CDCl_3$) -138.4 (s); *m/z* (ESI) 274 $[M+H]^+$ (Found: $[M+H]^+$, 274.0874. $C_{13}H_8NO_3F$ requires $[M+H]^+$, 274.0874); HPLC analysis: t_{R1} = 15.649 min, t_{R2} = 18.993 min, t_{R3} = 34.996 min.

Ethyl 2-(2-fluoro-3-methoxyphenyl)-4-methoxy-6-methylnicotinate



To a mixture of 2-fluoro-3-methoxyphenylboronic acid **243** (765 mg, 4.50 mmol, 1.1 equiv.), **260** (940 mg, 4.09 mmol, 1.0 equiv.), tetrakis(triphenylphosphine)palladium(0) (473 mg, 0.41 mmol, 0.1 equiv.) was added tetrahydrofuran (20 mL). The mixture was stirred at 80 ºC for 1 h, then potassium carbonate solution (2 M aqueous solution; 5.12 mL, 10.24 mmol, 2.5 equiv.) was added and the resulting mixture stirred at 80 ºC for 25 h. The reaction solution was cooled to rt and diluted with dichloromethane (500 mL) and water (50 mL). The layers were separated and the organic phase washed with brine (50 mL). The combined aqueous phases were extracted with dichloromethane (3 × 500 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (44.4% ethyl acetate-hexane) afforded ethyl 2-(2-fluoro-3-methoxyphenyl)-4-methoxy-6-methylnicotinate **261** (1.13 g, 86%) as a colourless oil; R_f 0.24 (44.4% ethyl acetate-hexane); ν_{max} (neat) 2943, 1720 (C=O), 1575, 1484, 1351, 1274, 1207, 1040, 790, 765 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.11-7.05 (1H, m, H-6'), 6.99-6.94 (2H, m, H-4', H-5'), 6.74 (1H, s, H-5), 4.10 (2H, q, J 7.1 Hz, O-C$\underline{H}_2$-CH_3), 3.90 (s, 3H, 3'-O-C$\underline{H}_3$), 3.87 (s, 3H, 4-O-C$\underline{H}_3$), 2.58 (s, 3H, 6-C$\underline{H}_3$), 0.99 (3H, t, J 7.1 Hz, O-CH_2-C$\underline{H}_3$); δ_c (100 MHz, $CDCl_3$) 166.8 (C=O), 166.0 (C-4), 163.8 (C-6), 161.5 (d, $^2J_{CF}$ 14.5 Hz, C-3'), 153.0 (C-2), 149.8 (d, $^1J_{CF}$ 248.2 Hz, C-2'), 128.3 (d, $^3J_{CF}$ 12.7 Hz, C-6'), 123.9 (d, $^3J_{CF}$ 11.3 Hz, C-4'), 122.2 (d, $^4J_{CF}$ 1.9 Hz, C-5'), 117.7 (d, $^2J_{CF}$ 23.1 Hz, C-1'), 113.9 (C-5), 105.2 (C-3), 61.3 (O-C$\underline{H}_2$-CH_3), 56.5 (3'-O-C$\underline{H}_3$), 56.0 (4'-O-C$\underline{H}_3$), 25.3 (6-C$\underline{H}_3$), 13.7 (O-CH_2-C$\underline{H}_3$); δ_F (376 MHz, $CDCl_3$) -138.9 (s); *m/z* (ESI) 320 $[M+H]^+$ (Found: $[M+H]^+$, 320.1289. $C_{17}H_{18}NO_4F$ requires $[M+H]^+$, 320.1298).

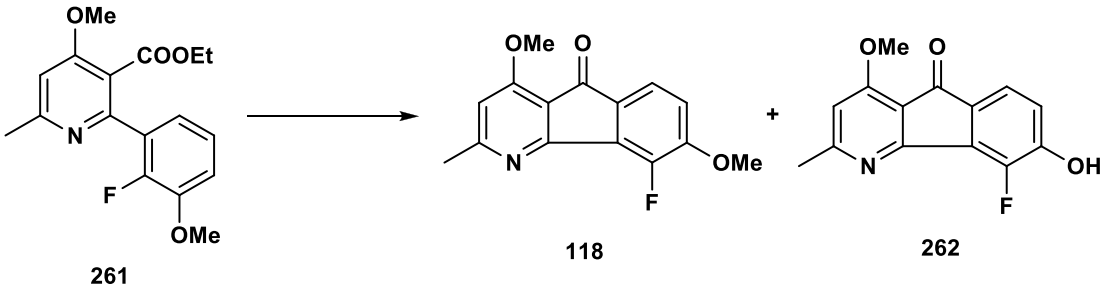
Methyl 2-chloro-6-methylnicotinate



256 **257**

To a solution of 2-chloro-6-methylnicotinic acid **256** (5.67 g, 33.0 mmol, 1.0 equiv.) in dry *N*,*N*-dimethylformamide (49 mL) was added anhydrous potassium carbonate (6.80 g, 49.6 mmol, 1.5 equiv.) and iodomethane (3.1 mL, 49.6 mmol, 1.5 equiv.) at 0 ºC. The mixture was stirred at rt for 18 h. After removal of the solvent *in vacuo*, the residue was partitioned between dichloromethane (450 mL) and water (50 mL). The aqueous phase was extracted with dichloromethane (2 × 450 mL). The combined organic phases were dried (Na_2SO_4). Concentration under reduced pressure and chromatography (11.8% ethyl acetate-hexane) afforded 2-chloro-6-methylnicotinate (6.02 g, 98%) as a white solid; R_f 0.36 (16.7% ethyl acetate-hexane); mp 138-140 °C; ν_{max} (solid) 2974, 1733 (C=O), 1561, 1354, 1280, 1109, 1058, 867 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.09 (1H, d, J 7.9 Hz, H-4), 7.16 (1H, d, J 7.9 Hz, H-5), 3.94 (3H, s, O-C$\underline{H}_3$), 2.59 (s, 3H, 6-C$\underline{H}_3$); δ_c (100 MHz, $CDCl_3$) 165.1 (C=O), 162.6 (C-6), 149.6 (C-2), 140.9 (C-4), 123.6 (C-3), 121.8 (C-5), 52.8 (O-$\underline{C}H_3$), 24.5 (6-$\underline{C}H_3$); *m/z* (ESI) 186 $[M+H]^+$. *In agreement with published data*.[151#]

1-Methoxy-5-fluoro-3-methyl-6-methoxy-4-azafluoren-9-one

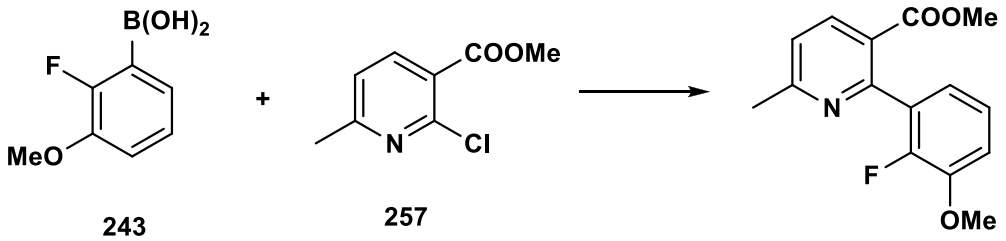


A mixture of ethyl 2-(2-fluoro-3-methoxyphenyl)-4-methoxy-6-methylnicotinate **261** (741 mg, 2.32 mmol, 1.0 equiv.) and polyphosphoric acid (11.6 g, 5.8 mL) was stirred at 130 ºC for 11 h. The mixture was poured on crushed ice, neutralised with saturated potassium hydroxide solution and partitioned between chloroform (1 L) and water. The aqueous phase was extracted with chloroform (3 × 1 L) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (1% methanol-dichloromethane) afforded 1-methoxy-5-fluoro-3-methyl-6-methoxy-4-azafluoren-9-one **118** (173 mg, 27%) as a yellow solid and 1-methoxy-3-methyl-5-fluoro-6-hydroxy-4-azafluoren-9-one **262** (211 mg, 35%) as a yellow solid.

1-Methoxy-5-fluoro-3-methyl-6-methoxy-4-azafluoren-9-one **118**. R_f 0.52 (3.2% methanol-dichloromethane); mp 212-213 °C; ν_{max} (solid) 2948, 1701 (C=O), 1577, 1504, 1375, 1276, 1213, 1033, 858, 790, 772 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.00 (d, 1H, J 8.0 Hz, H-8), 6.97 (t, 1H, J 8.0 Hz, H-7), 6.61 (s, 1H, H-2), 4.00 (s, 3H, 6-O-C$\underline{H}_3$), 3.96 (s, 3H, 1-O-C$\underline{H}_3$), 2.64 (s, 3H, 3-C$\underline{H}_3$); δ_c (100 MHz, $CDCl_3$) 188.3 (C=O), 167.2 (C-1), 165.0 (C-4a), 163.4 (C-3), 154.2 (d, $^2J_{CF}$ 10.3 Hz, C-6), 147.0 (d, $^1J_{CF}$ 262.0 Hz, C-5), 128.8 (d, $^2J_{CF}$ 8.8 Hz, C-4b), 128.7 (d, $^3J_{CF}$ 7.3 Hz, C-8a), 120.7 (d, $^3J_{CF}$ 3.5 Hz, C-7), 114.4 (C-9a), 113.6 (d, $^4J_{CF}$ 1.0 Hz, C-8), 106.9 (C-2), 56.9 (6-O-$\underline{C}H_3$), 56.1 (1-O-$\underline{C}H_3$), 25.8 (1-$\underline{C}H_3$); δ_F (376 MHz, $CDCl_3$) -137.4 (s); *m/z* (ESI) 274 $[M+H]^+$ (Found: $[M+H]^+$, 274.0874. $C_{15}H_{12}NO_3F$ requires $[M+H]^+$, 274.0880); HPLC analysis: t_{R1} = 15.559 min, t_{R2} = 18.884 min, t_{R3} = 34.308 min.

1-Methoxy-3-methyl-5-fluoro-6-hydroxy-4-azafluoren-9-one **262**. R_f 0.12 (3.2% methanol-dichloromethane); mp 282-283 °C; ν_{max} (solid) 3289, 2923, 1691 (C=O), 1579, 1378, 1237, 1210, 996, 846, 792 cm^{-1}; δ_H (400 MHz, DMSO-*d*$_6$) 7.21 (d, 1H, J 7.9 Hz, H-8), 6.92-6.82 (m, 2H, H-7, H-2), 3.86 (s, 3H, 3-O-C$\underline{H}_3$), 2.44 (s, 3H, 1-C$\underline{H}_3$); δ_c (100 MHz, DMSO-*d*$_6$) 187.1 (C=O), 166.3 (C-1), 163.8 (C-4a), 162.8 (C-3),152.4 (d, $^2J_{CF}$ 11.3 Hz, C-6), 147.0 (d, $^1J_{CF}$ 257.8 Hz, C-5), 128.6 (d, $^2J_{CF}$ 8.7 Hz, C-4b), 126.5 (d, $^3J_{CF}$ 3.8 Hz, C-8a), 120.6 (d, $^3J_{CF}$ 2.1 Hz, C-7), 118.4 (d, $^4J_{CF}$ 1.5 Hz, C-8), 113.1 (C-9a), 107.7 (C-2), 56.0 (1-O-$\underline{C}H_3$), 24.9 (1-$\underline{C}H_3$); δ_F (376 MHz, DMSO-*d*$_6$) -139.3 (s); *m/z* (ESI) 260 $[M+H]^+$ (Found: $[M+H]^+$, 260.0716. $C_{14}H_{10}NO_3F$ requires $[M+H]^+$, 260.0723); HPLC analysis: t_{R1} = 14.278 min, t_{R2} = 16.881 min, t_{R3} = 28.998 min.

Ethyl 2-(2-fluoro-3-methoxyphenyl)-6-methylnicotinate

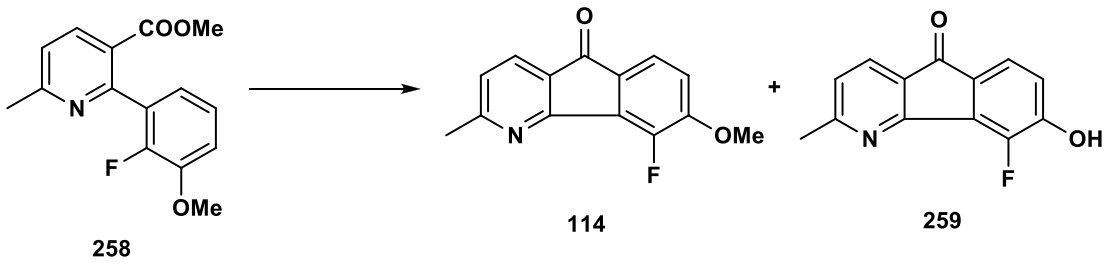


243 **257** **258**

To a mixture of 2-fluoro-3-methoxyphenylboronic acid **243** (767 mg, 4.51 mmol, 1.1 equiv.), **257** (761 mg, 4.10 mmol, 1.0 equiv.), tetrakis(triphenylphosphine)palladium(0) (473.8 mg, 0.41 mmol, 0.1 equiv.) was added tetrahydrofuran (20 mL). The mixture

was stirred at 80 ºC for 1 h, then potassium carbonate solution (2 M aqueous solution; 5.12 mL, 10.24 mmol, 2.5 equiv.) was added and the resulting mixture stirred at 80 ºC for 24 h. The reaction solution was cooled to rt and diluted with dichloromethane (500 mL) and water (50 mL). The layers were separated and the organic phase washed with brine (50 mL). The combined aqueous phases were extracted with dichloromethane (3 × 500 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (16.7% ethyl acetate-hexane) afforded ethyl 2-(2-fluoro-3-methoxyphenyl)-6-methylnicotinate **258** (974 mg, 86%) as a colourless oil; R_f 0.28 (16.7% ethyl acetate-hexane); ν_{max} (neat) 2959, 1727 (C=O), 1580, 1432, 1269, 1136, 1082, 1042, 777, 730 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.12 (1H, d, J 8.0 Hz, H-6), 7.20 (1H, d, J 8.0 Hz, H-5), 7.17-7.07 (2H, m, H-4', H-5'), 7.00 (1H, td, J 8.0, 1.6 Hz, H-5'), 3.87 (s, 3H, COOC$\underline{H}_3$), 3.72 (s, 3H, 3'-O-C$\underline{H}_3$), 2.62 (s, 3H, 6-C$\underline{H}_3$); δ_c (100 MHz, $CDCl_3$) 166.8 (C=O), 161.7 (C-6), 153.5 (C-2), 149.7 (d, $^1J_{CF}$ 246.3 Hz, C-2'), 147.3 (d, $^2J_{CF}$ 11.1 Hz, C-3'), 138.3 (C-4), 129.3 (d, $^2J_{CF}$ 12.6 Hz, C-1'), 124.5 (C-3), 124.0 (d, $^3J_{CF}$ 4.6 Hz, C-6'), 122.1 (C-5), 122.0 (d, $^4J_{CF}$ 2.2 Hz, C-5'), 113.7 (d, $^3J_{CF}$ 2.0 Hz, C-4'), 56.4 (3'-O-$\underline{C}H_3$), 52.2 (COO$\underline{C}H_3$), 24.8 (6-$\underline{C}H_3$); δ_F (376 MHz, $CDCl_3$) -140.0 (s); *m/z* (ESI) 276 $[M+H]^+$ (Found: $[M+H]^+$, 276.1028. $C_{15}H_{14}NO_3F$ requires $[M+H]^+$, 276.1036).

5-Fluoro-3-methyl-6-methoxy-4-azafluoren-9-one



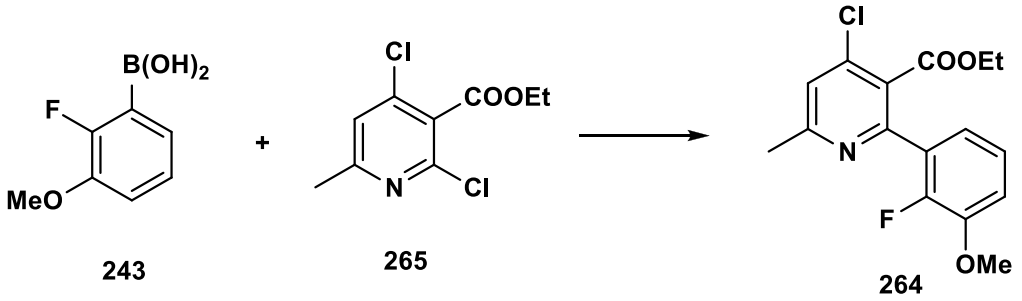
258 114 259

A mixture of ethyl 2-(2-fluoro-3-methoxyphenyl)-6-methylnicotinate **258** (694 mg, 2.52 mmol, 1.0 equiv.) and polyphosphoric acid (12.6 g, 6.3 mL) was stirred at 130 ºC for 11 h. The mixture was poured on crushed ice, neutralised with saturated potassium hydroxide solution and partitioned between chloroform (1 L) and water. The aqueous phase was extracted with chloroform (3 × 1 L) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (0.66% methanol-dichloromethane) afforded 5-fluoro-3-methyl-6-methoxy-4- azafluoren-9-one **114** (178 mg, 29%) as a yellow solid and 5-fluoro-3-methyl-6-hydroxy-4-azafluoren-9-one **259** (141 mg, 24%) as a yellow solid.

5-Fluoro-3-methyl-6-methoxy-4-azafluoren-9-one **114**. R_f 0.68 (2.4% methanol-dichloromethane); mp 159-160 °C; ν_{max} (solid) 2958, 1716 (C=O), 1577, 1502, 1394, 1279, 1117, 846, 790, 769, 693 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.78 (d, 1H, J 7.7 Hz, H-1), 7.49 (d, 1H, J 8.1 Hz, H-8), 7.08 (d, 1H, J 7.7 Hz, H-2), 6.97 (t, 1H, J 8.0 Hz, H-7), 3.97 (s, 3H, O-C$\underline{H}_3$), 2.67 (s, 3H, 3-C$\underline{H}_3$); δ_c (100 MHz, $CDCl_3$) 189.8 (C=O), 165.0 (C-4a), 161.8 (C-3), 154.8 (d, $^2J_{CF}$ 10.2 Hz, C-6), 149.9 (d, $^1J_{CF}$ 246.4 Hz, C-5), 138.6 (d, $^2J_{CF}$ 20.8 Hz, C-4b), 132.2 (d, $^3J_{CF}$ 10.0 Hz, C-8a), 131.6 (C-1), 128.7 (d, $^3J_{CF}$ 8.2 Hz, C-7), 127.1 (C-9a), 122.9 (C-2), 121.3 (d, $^4J_{CF}$ 3.5 Hz, C-8), 25.4 (3-$\underline{C}H_3$); δ_F (376 MHz, $CDCl_3$) -137.2 (s); *m/z* (ESI) 244 $[M+H]^+$ (Found: $[M+H]^+$, 244.0769. $C_{14}H_{10}NO_2F$ requires $[M+H]^+$, 244.0774).

5-Fluoro-3-methyl-6-hydroxy-4-azafluoren-9-one **259**. R_f 0.17 (2.4% methanol-dichloromethane); mp 212-213 °C; ν_{max} (solid) 3237, 1719 (C=O), 1579, 1478, 1385, 1269, 1070, 972, 786, 726, 683 cm^{-1}; δ_H (400 MHz, $CDCl_3$ + CD_3OD) 7.94 (d, 1H, J 8.0 Hz, H-1), 7.55 (d, 1H, J 7.7 Hz, H-8), 6.90 (d, 1H, J 7.7 Hz, H-2), 6.73 (t, 1H, J 7.7 Hz, H-7), 2.40 (s, 3H, 3-C$\underline{H}_3$); δ_c (100 MHz, $CDCl_3$) 186.8 (C=O), 165.3 (C-3), 164.1 (C-4a), 154.7 (d, $^2J_{CF}$ 10.0 Hz, C-6), 147.1 (d, $^1J_{CF}$ 263.3 Hz, C-5), 141.6 (C-1), 128.5 (d, $^3J_{CF}$ 4.8 Hz, C-8a), 128.2 (d, $^2J_{CF}$ 8.9 Hz, C-4b), 124.2 (C-2), 123.3 (C-9a), 121.4 (d, $^3J_{CF}$ 3.6 Hz, C-7), 114.2 (d, $^4J_{CF}$ 1.2 Hz, C-8), 56.9 (O-$\underline{C}H_3$), 25.0 (3-$\underline{C}H_3$); δ_F (376 MHz, $CDCl_3$) -136.5 (s); *m/z* (ESI) 230 $[M+H]^+$ (Found: $[M+H]^+$, 230.0611. $C_{13}H_8NO_2F$ requires $[M+H]^+$, 230.0617).

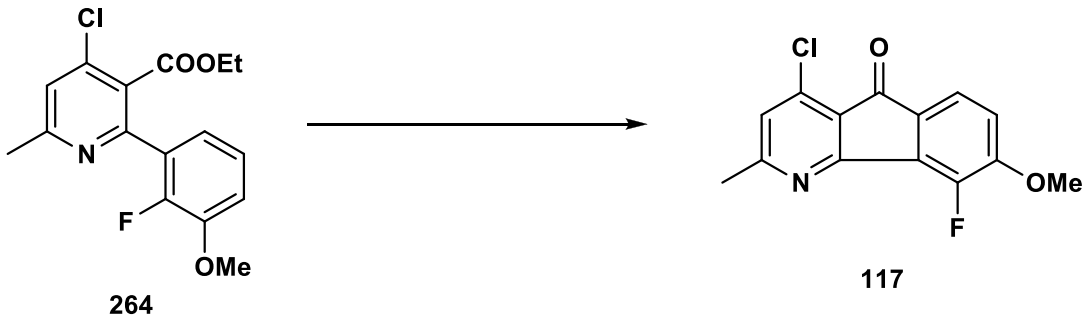
Ethyl 4-chloro-2-(2-fluoro-3-methoxyphenyl)-6-methylnicotinate



To a mixture of **243** (8.00 g, 34.07 mmol, 1.0 equiv.), tetrakis(triphenylphosphine)palladium(0) (1.77 g, 1.54 mmol, 0.045 equiv.) and cesium carbonate (19.99 g, 61.38 mmol, 1.8 equiv.) was added acetonitrile (110 mL) and water (22 mL). The mixture was stirred at rt for 10 min, then 2-fluoro-3-methoxyphenylboronic acid **243** (6.37 g, 37.48 mmol, 1.1 equiv.) in dimethoxyethane (110 mL) was added and the resulting mixture stirred at 80 ºC for 16 h. The reaction solution was cooled to rt and diluted with dichloromethane (1 L) and

water (100 mL). The layers were separated and the organic phase washed with brine (50 mL). The combined aqueous phases were extracted with dichloromethane (3×500 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (14.3% acetone-hexane) afforded ethyl 4-chloro-2-(2-fluoro-3-methoxyphenyl)-6-methylnicotinate **264** (9.15 g, 83%) as a white solid; R_f 0.30 (14.3% acetone-hexane); mp 95-96 °C; ν_{max} (solid) 2972, 1732 (C=O), 1570, 1488, 1370, 1273, 1146, 1078, 859, 785, 753 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.29 (1H, s, H-5), 7.14-7.11 (1H, m, H-6'), 7.05-6.97 (2H, m, H-4', H-5'), 4.19 (2H, q, J 7.1 Hz, O-C$\underline{H}_2$-CH_3), 3.90 (s, 3H, O-C$\underline{H}_3$), 2.62 (s, 3H, C$\underline{H}_3$), 1.09 (3H, t, J 7.1 Hz, O-CH_2-C$\underline{H}_3$); δ_c (100 MHz, $CDCl_3$) 165.3 (C=O), 160.4 (C-6), 153.2 (C-2), 149.8 (d, $^1J_{CF}$ 248.8 Hz, C-2'), 147.9 (d, $^2J_{CF}$ 10.7 Hz, C-3'), 142.1 (C-4), 127.7 (d, $^2J_{CF}$ 12.6 Hz, C-1'), 127.3 (C-3), 124.0 (d, $^3J_{CF}$ 4.8 Hz, C-6'), 123.3 (C-5), 122.1 (d, $^4J_{CF}$ 1.6 Hz, C-5'), 114.2 (d, $^3J_{CF}$ 1.9 Hz, C-4'), 61.9 (O-$\underline{C}H_2$-CH_3), 56.5 (O-$\underline{C}H_3$), 24.5 (6-$\underline{C}H_3$), 13.7 (O-CH_2-$\underline{C}H_3$); δ_F (376 MHz, $CDCl_3$) -138.3 (s); *m/z* (ESI) 324 $[M+H]^+$ (Found: $[M+H]^+$, 324.0798. $C_{16}H_{15}NO_3FCl$ requires $[M+H]^+$, 324.0797).

1-Chloro-5-fluoro-3-methyl-6-methoxy-4-azafluoren-9-one

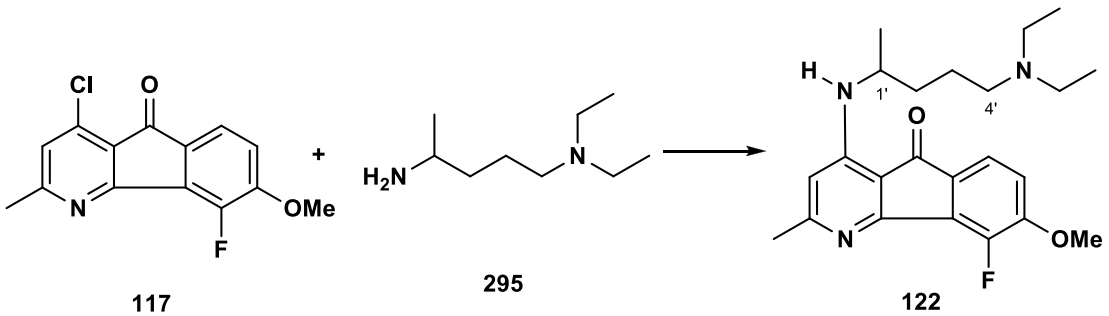


264 **117**

A mixture of ethyl 4-chloro-2-(2-fluoro-3-methoxyphenyl)-6-methylnicotinate **164** (3.00 g, 10.8 mmol) and Eaton's reagent (21 mL) was stirred at 110 ºC for 30 h. Then reaction mixture was cooled to rt and crushed ice was added. The resulting mixture was

neutralised with saturated potassium hydroxide solution and partitioned between chloroform (1 L) and water. The aqueous phase was extracted with chloroform and tetrahydrofuran (3:1, 3 × 1 L) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (13.3% acetone-hexane) afforded 1-chloro-5-fluoro-3-methyl-6-methoxy-4-azafluoren-9-one **117** (1.42 g, 55%) as a yellow solid; R_f 0.36 (14.3% acetone-hexane); mp 204-205 °C; ν_{max} (solid) 2954, 1712 (C=O), 1575, 1505, 1373, 1286, 1242, 1056, 835, 789, 760 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.51 (dd, 1H, J 8.0, 0.4 Hz, H-8), 7.03 (s, 1H, H-2), 6.93 (dd, 1H, J 8.0, 7.1 Hz, H-7), 3.98 (s, 3H, O-C<u>H</u>$_3$), 2.64 (s, 3H, 3-C<u>H</u>$_3$); δ_c (100 MHz, $CDCl_3$) 186.8 (C=O), 165.3 (C-3), 164.1 (C-4a), 154.7 (d, $^2J_{CF}$ 10.0 Hz, C-6), 147.1 (d, $^1J_{CF}$ 263.3 Hz, C-5), 141.6 (C-1), 128.5 (d, $^3J_{CF}$ 4.8 Hz, C-8a), 128.2 (d, $^2J_{CF}$ 8.9 Hz, C-4b), 124.2 (C-2), 123.3 (C-9a), 121.4 (d, $^3J_{CF}$ 3.6 Hz, C-7), 114.2 (d, $^4J_{CF}$ 1.2 Hz, C-8), 56.9 (O-<u>C</u>H$_3$), 25.0 (3-<u>C</u>H$_3$); δ_F (376 MHz, $CDCl_3$) -136.4 (s); *m/z* (ESI) 278 $[M+H]^+$ (Found: $[M+H]^+$, 278.0381. $C_{14}H_9NO_2FCl$ requires $[M+H]^+$, 278.0379); HPLC analysis: t_{R1} = 16.120 min, t_{R2} = 19.989 min, t_{R3} = 37.818 min.

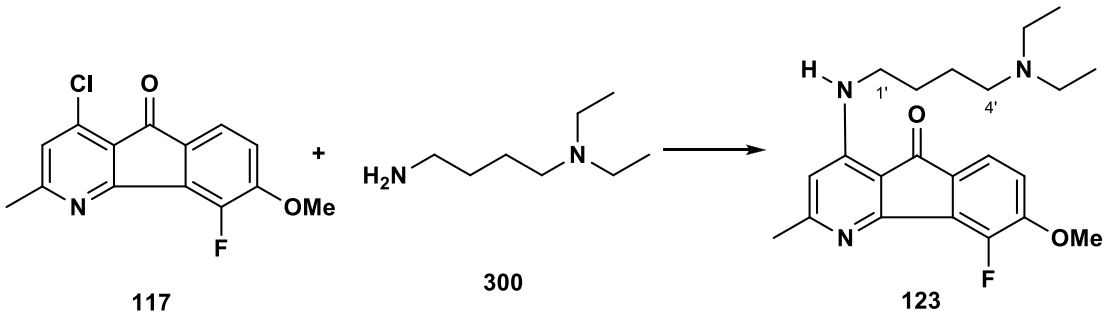
1-(4-Diethylamino-1-methyl-butylamino)-5-fluoro-3-methyl-6-methoxy-4-azafluoren-9-one



117 295 122

A mixture of 1-chloro-5-fluoro-3-methyl-6-methoxy-4-azafluoren-9-one **117** (100 mg, 0.36 mmol, 1.0 equiv.), **295** (418 µL, 2.16 mmol, 6.0 equiv.), phenol (203 mg, 2.16 mmol, 6.0 equiv.) and sodium iodide (10 mg) was stirred at 160 ºC for 2 h. After being cooled to rt, sodium hydroxide solution (1 M, 20 mL) was added to the reaction mixture. The mixture was extracted with chloroform (3 × 250 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and aluminium oxide column chromatography (0.5% methanol-dichloromethane) afforded 1-(4-diethylamino-1-methyl-butylamino)-5-fluoro-3-methyl-6-methoxy-4-azafluoren-9-one **122** (48 mg, 33%) as a yellow oil; R_f 0.19 (0.25% methanol-dichloromethane, aluminium oxide TLC plate); ν_{max} (neat) 3358, 2968, 1680 (C=O), 1598, 1582, 1495, 1371, 1277, 1238, 1033, 790, 775 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.36 (d, 1H, J 8.0 Hz, H-8), 6.84-6.76 (m, 2H, H-7, N<u>H</u>), 6.28 (s, 1H, H-2), 3.94 (s, 3H, O-C<u>H</u>$_3$), 3.71-3.62 (m, 1H, H-1'), 2.59 (4H, q, J 7.2 Hz, N-(C<u>H</u>$_2$-CH$_3$)$_2$), 2.52-2.44 (m, 5H, H-4', 3-C<u>H</u>$_3$), 1.63-1.57 (m, 4H, H-2', H-3'), 1.26 (d, 3H, J 6.4 Hz, 1'-C<u>H</u>$_3$), 1.05 (6H, t, J 7.2 Hz, N-(CH$_2$-C<u>H</u>$_3$)$_2$); δ_c (100 MHz, $CDCl_3$) 192.0 (C=O), 164.6 (C-3), 164.2 (C-4a), 153.8 (d, $^2J_{CF}$ 10.6 Hz, C-6), 151.3 (C-1), 147.1 (d, $^1J_{CF}$ 261.2 Hz, C-5), 129.3 (d, $^3J_{CF}$ 4.3 Hz, C-8a), 128.6 (d, $^2J_{CF}$ 9.0 Hz, C-4b), 119.6 (d, $^3J_{CF}$ 3.2 Hz, C-7), 112.9 (C-8), 109.0 (C-9a), 105.0 (C-2), 56.8 (O-<u>C</u>H$_3$), 52.5 (C-4'), 47.7 (C-1'), 46.8 (N-(<u>C</u>H$_2$-CH$_3$)$_2$), 34.9 (C-2'), 25.8 (C-3'), 23.2 (1'-<u>C</u>H$_3$), 21.1 (3-<u>C</u>H$_3$), 11.2 (N-(CH$_2$-<u>C</u>H$_3$)$_2$); δ_F (376 MHz, $CDCl_3$) -137.4 (s); *m/z* (ESI) 400 $[M+H]^+$ (Found: $[M+H]^+$, 400.2390. $C_{23}H_{30}N_3O_2F$ requires $[M+H]^+$, 400.2400); HPLC analysis: t_{R1} = 13.586 min, t_{R2} = 15.755 min, t_{R3} = 25.306 min.

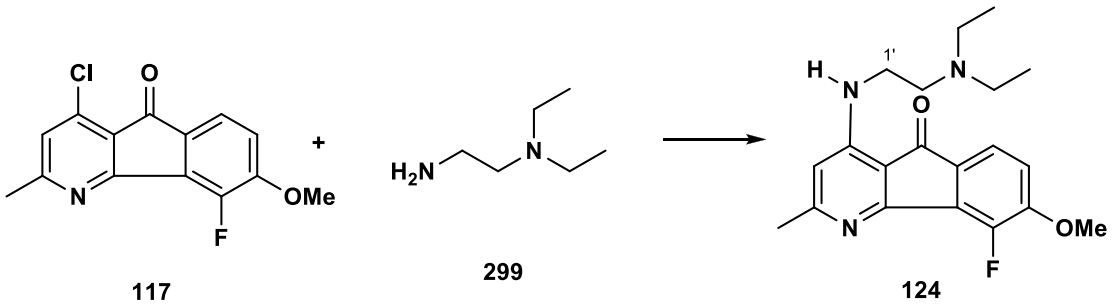
1-(4-Diethylamino-butylamino)-5-fluoro-6-methoxy-3-methyl-4-azafluoren-9-one



117 300 123

A mixture of 1-chloro-5-fluoro-3-methyl-6-methoxy-4-azafluoren-9-one **117** (150 mg, 0.54 mmol, 1.0 equiv.), **300** (376 µL, 2.16 mmol, 4.0 equiv.), phenol (203 mg, 2.16 mmol, 4.0 equiv.) and sodium iodide (15 mg) was stirred at 130 ºC for 2 h. After being cooled to rt, sodium hydroxide solution (1 M, 20 mL) was added to the reaction mixture. The mixture was extracted with chloroform (3 × 250 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and aluminium oxide column chromatography (0.66% methanol-dichloromethane) afforded 1-(4-diethylamino-butylamino)-5-fluoro-6-methoxy-3-methyl-4-azafluoren- 9-one **123** (140 mg, 67%) as a yellow oil; R_f 0.31 (1% methanol-dichloromethane, aluminium oxide TLC plate); ν_{max} (neat) 3381, 2970, 1675 (C=O), 1602, 1579, 1495, 1280, 1231, 1044, 986, 789, 776 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.35 (d, 1H, J 7.6 Hz, H-8), 6.90 (t, 1H, J 5.6 Hz, N<u>H</u>), 6.81 (t, 1H, J 7.6 Hz, H-7), 6.27 (s, 1H, H-2), 3.93 (s, 3H, O-C<u>H</u>$_3$), 3.32-3.21 (m, 2H, H-1'), 2.54-2.43 (m, 9H, H-4', N-(C<u>H</u>$_2$-CH_3)$_2$, 3-C<u>H</u>$_3$), 1.72-1.62 (m, 2H, H-3'), 1.59-1.50 (m, 2H, H-3'), 1.00 (6H, t, J 7.1 Hz, N-(CH_2-C<u>H</u>$_3$)$_2$); δ_c (100 MHz, $CDCl_3$) 192.0 (C=O), 164.6 (C-3), 164.0 (C-4a), 153.8 (d, $^2J_{CF}$ 10.5 Hz, C-6), 151.9 (C-1), 147.1 (d, $^1J_{CF}$ 261.2 Hz, C-5), 129.3 (d, $^3J_{CF}$ 2.1 Hz, C-8a), 128.7 (d, $^2J_{CF}$ 9.0 Hz, C-4b), 119.6 (d, $^3J_{CF}$ 3.3 Hz, C-7), 112.9 (C-8), 109.1 (C-9a), 104.8 (C-2), 56.8 (O-<u>C</u>H_3), 52.5 (C-4'), 46.9 (N-(<u>C</u>H_2-CH_3)$_2$), 42.1 (C-1'), 27.5 (C-2'), 25.7 (C-3'), 24.6 (3-<u>C</u>H_3), 11.8 (N-(CH_2-<u>C</u>H_3)$_2$); δ_F (376 MHz, $CDCl_3$) -137.4 (s); *m/z* (ESI) 386 $[M+H]^+$ (Found: $[M+H]^+$, 386.2227. $C_{22}H_{28}N_3O_2F$ requires $[M+H]^+$, 386.2244); HPLC analysis: t_{R1} = 13.134 min, t_{R2} = 15.240 min, t_{R3} = 24.672 min.

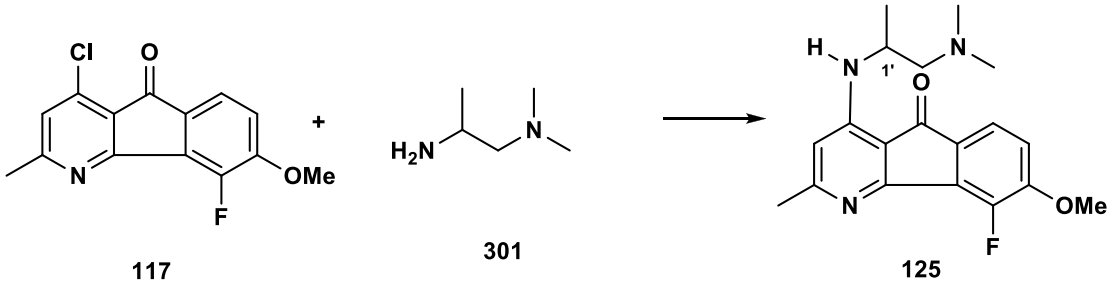
1-(4-Diethylamino-ethylamino)-5-fluoro-6-methoxy-3-methyl-4- azafluoren-9-one



117 299 124

A mixture of 1-chloro-5-fluoro-3-methyl-6-methoxy-4-azafluoren-9-one **117** (100 mg, 0.36 mmol, 1.0 equiv.), **299** (303 µL, 2.16 mmol, 6.0 equiv.), phenol (203 mg, 2.16 mmol, 6.0 equiv.) and sodium iodide (10 mg) was stirred at 130 ºC for 2.5 h. After being cooled to rt, sodium hydroxide solution (1 M, 20 mL) was added to the reaction mixture. The mixture was extracted with chloroform (3 × 250 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and aluminium oxide column chromatography (0.4% methanol-dichloromethane) afforded 1-(4-diethylamino-ethylamino)-5-fluoro- 6-methoxy-3-methyl-4-azafluoren-9-one **124** (60 mg, 47%) as a yellow oil; R_f 0.26 (0.4% methanol-dichloromethane, aluminium oxide TLC plate); ν_{max} (neat) 3365, 2966, 1683 (C=O), 1560, 1495, 1371, 1276, 1233, 1051, 987, 790, 772 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.38 (d, 1H, J 7.6 Hz, H-8), 7.35 (t, 1H, J 4.8 Hz, N<u>H</u>), 6.83 (t, 1H, J 7.6 Hz, H-7), 6.27 (s, 1H, H-2), 3.94 (s, 3H, O-C<u>H</u>$_3$), 3.32-3.23 (m, 2H, H-1'), 2.71 (t, 2H, J 6.2 Hz, H-2'), 2.59 (q, 4H, J 7.1 Hz, N-(C<u>H</u>$_2$-CH$_3$)$_2$), 2.53 (s, 3H, 3-C<u>H</u>$_3$), 1.08 (6H, t, J 7.1 Hz, N-(CH$_2$-C<u>H</u>$_3$)$_2$); δ_c (100 MHz, $CDCl_3$) 191.8 (C=O), 164.5 (C-3), 164.1 (C-4a), 153.8 (d, $^2J_{CF}$ 10.6 Hz, C-6), 151.6 (C-1), 147.1 (d, $^1J_{CF}$ 261.0 Hz, C-5), 129.4 (d, $^3J_{CF}$ 1.9 Hz, C-8a), 128.8 (d, $^2J_{CF}$ 9.1 Hz, C-4b), 119.6 (d, $^3J_{CF}$ 3.2 Hz, C-7), 113.0 (C-8), 109.3 (C-9a), 105.2 (C-2), 56.8 (O-<u>C</u>H$_3$), 51.2 (C-2'), 47.1 (N-(<u>C</u>H$_2$-CH$_3$)$_2$), 39.9 (C-1'), 25.8 (3-<u>C</u>H$_3$), 12.2 (N-(CH$_2$-<u>C</u>H$_3$)$_2$); δ_F (376 MHz, $CDCl_3$) -137.6 (s); *m/z* (ESI) 358 $[M+H]^+$ (Found: $[M+H]^+$, 358.1925. $C_{20}H_{24}N_3O_2F$ requires $[M+H]^+$, 358.1931); HPLC analysis: t_{R1} = 13.872 min, t_{R2} = 16.115 min, t_{R3} = 25.446 min.

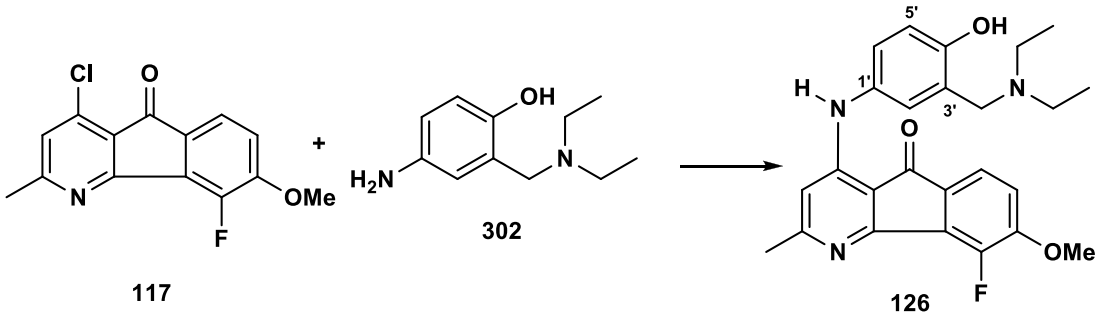
1-(4-Dimethylamino-1-methyl-ethylamino)-5-fluoro-6-methoxy-3-methyl-4-azafluoren-9-one



117 301 125

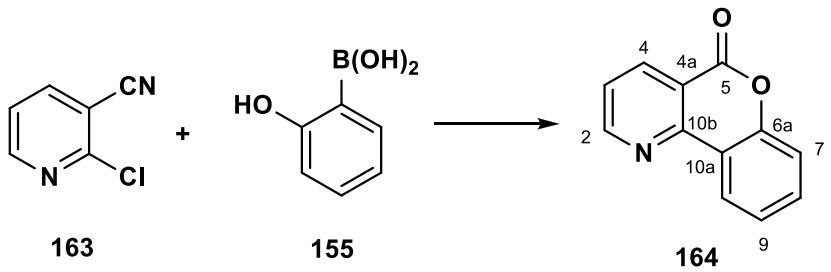
A mixture of 1-chloro-5-fluoro-3-methyl-6-methoxy-4-azafluoren-9-one **117** (150 mg, 0.54 mmol, 1.0 equiv.), **301** (418 µL, 3.24 mmol, 6.0 equiv.), phenol (686 mg, 7.29 mmol, 13.5 equiv.) and sodium iodide (30 mg) was stirred at 100 ºC for 2 h. After being cooled to rt, sodium hydroxide solution (1 M, 20 mL) was added to the reaction mixture. The mixture was extracted with chloroform (3 × 250 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and aluminium oxide column chromatography (11.1% acetone-hexane) afforded **125** (112 mg, 60%) as a yellow oil; R_f 0.13 (10% acetone-hexane, aluminium oxide TLC plate); ν_{max} (neat) 3361, 2946, 1681 (C=O), 1596, 1582, 1494, 1369, 1275, 1237, 1071, 1027, 988, 789, 775 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.38 (d, 1H, J 7.6 Hz, H-8), 7.01 (d, 1H, J 7.4 Hz, N<u>H</u>), 6.83 (t, 1H, J 7.6 Hz, H-7), 6.31 (s, 1H, H-2), 3.94 (s, 3H, O-C<u>H</u>$_3$), 3.78-3.72 (m, 1H, H-1'), 2.53 (s, 3H, 3-C<u>H</u>$_3$), 2.51-2.48 (m, 1H, H-2'a), 2.41-2.37 (m, 1H, H-2'b), 2.32 (s, 6H, N-(C<u>H</u>$_3$)$_2$), 1.28 (d, 3H, J 6.4 Hz, 1'-C<u>H</u>$_3$); δ_c (100 MHz, $CDCl_3$) 192.0 (C=O), 164.6 (C-3), 164.2 (C-4a), 153.8 (d, $^2J_{CF}$ 10.6 Hz, C-6), 151.3 (C-1), 147.1 (d, $^1J_{CF}$ 261.2 Hz, C-5), 129.3 (C-8a), 128.7 (d, $^2J_{CF}$ 9.0 Hz, C-4b), 119.6 (d, $^3J_{CF}$ 3.4 Hz, C-7), 113.0 (C-8), 109.4 (C-9a), 105.1 (C-2), 65.0 (C-2'), 56.8 (O-<u>C</u>H$_3$), 46.0 (N-(<u>C</u>H$_3$)$_2$), 42.9 (C-1'), 25.8 (3-<u>C</u>H$_3$), 19.7 (1'-<u>C</u>H$_3$); δ_F (376 MHz, $CDCl_3$) – 137.4 (s); *m/z* (ESI) 344 $[M+H]^+$ (Found: $[M+H]^+$, 344.1769. $C_{19}H_{22}N_3O_2F$ requires $[M+H]^+$, 344.1774); HPLC analysis: t_{R1} = 11.601 min, t_{R2} = 12.867 min, t_{R3} = 17.711 min.

1-(3-Diethylaminomethyl-4-hydroxy-phenylamino)-5-fluoro-6-methoxy-3-methyl-4-azafluoren-9-one



A mixture of 1-chloro-5-fluoro-3-methyl-6-methoxy-4-azafluoren-9-one **117** (120 mg, 0.43 mmol, 1.0 equiv.), **302** (127 mg, 0.48 mmol, 1.1 equiv.) and 2-ethoxyethanol (14 mL) was stirred at 120 ºC for 6.5 h. After being cooled to rt, the mixture was adjusted to pH 9 with aqueous ammonia. The resulting mixture was extracted with chloroform (3×250 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and aluminium oxide column chromatography (12.5% acetone-hexane) afforded **126** (107 mg, 57%) as a yellow oil; R_f 0.19 (12.5% acetone-hexane, aluminium oxide TLC plate); ν_{max} (neat) 3347, 2971, 1681 (C=O), 1587, 1496, 1369, 1233, 1073, 988, 824, 790 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.41 (s, 1H, N<u>H</u>), 7.41 (d, 1H, J 7.6 Hz, H-8), 7.06 (dd, 1H, J 8.5, 2.6 Hz, H-6'), 6.87-6.82 (m, 3H, H-7, H-2', H-5'), 6.42 (s, 1H, H-2), 3.95 (s, 3H, O-C<u>H</u>$_3$), 3.78 (s, 2H, C<u>H</u>$_2$-N-(CH_2-CH_3)$_2$), 2.64 (q, 4H, J 7.2 Hz, N-(C<u>H</u>$_2$-CH_3)$_2$), 2.46 (s, 3H, 3-C<u>H</u>$_3$), 1.12 (6H, t, J 7.2 Hz, N-(CH_2-C<u>H</u>$_3$)$_2$); δ_c (100 MHz, $CDCl_3$) 191.9 (C=O), 164.7 (C-3), 164.1 (C-4a), 157.0 (C-1), 154.0 (d, $^2J_{CF}$ 10.5 Hz, C-6), 150.7 (C-4'), 147.1 (d, $^1J_{CF}$ 261.3 Hz, C-5), 129.2 (d, $^3J_{CF}$ 2.0 Hz, C-8a), 128.8 (d, $^2J_{CF}$ 9.0 Hz, C-4b), 128.5 (C-3'), 125.4 (d, $^3J_{CF}$ 18.4 Hz, C-7), 123.2 (C-2'), 119.9 (d, $^3J_{CF}$ 3.3 Hz, C-8), 117.0 (C-6'), 113.0 (C-5'), 109.4 (C-9a), 105.9 (C-2), 56.9 (O-<u>C</u>H_3), 56.8 (<u>C</u>H_2-N(C_2H_5)$_2$), 46.5 (N-(<u>C</u>H_2-CH_3)$_2$), 25.7 (3-<u>C</u>H_3), 11.3 (N-(CH_2-<u>C</u>H_3)$_2$); δ_F (376 MHz, $CDCl_3$) -137.1 (s); *m/z* (ESI) 436 $[M+H]^+$ (Found: $[M+H]^+$, 436.2038. $C_{25}H_{26}N_3O_3F$ requires $[M+H]^+$, 436.2036); HPLC analysis: t_{R1} = 10.968 min, t_{R2} = 12.224 min, t_{R3} = 15.176 min.

Pyrido[3,2-*c*]coumarin



163 **155** **164**

Thermal reaction conditions:

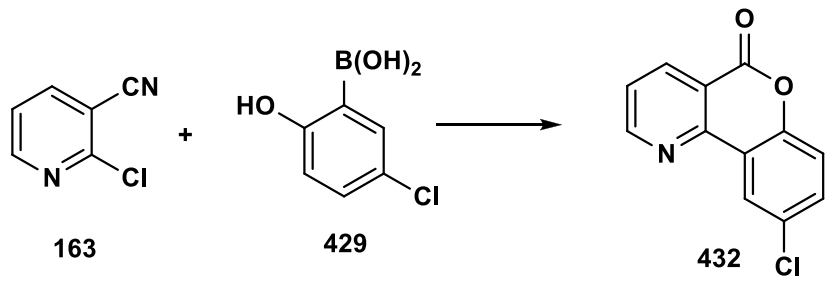
A mixture of 2-chloronicotinonitrile (70 mg, 0.51 mmol, 1.0 equiv.), 2-hydroxyphenylboronic acid (84 mg, 0.61 mmol, 1.2 equiv.), tetrakis(triphenylphosphine)palladium(0) (58 mg, 0.05 mmol, 0.1 equiv.) and 1,4-dioxane (5.0 mL) was stirred at 80 ºC for 1 h, then water (0.5 mL) and potassium carbonate solution (2 M aqueous solution; 1.0 mL, 1.02 mmol, 4.0 equiv.) were added at rt and the resulting mixture stirred at 80 ºC for 12 h. The mixture was diluted with dichloromethane (150 mL) and water (20 mL). The layers were separated and the organic phase washed with brine (20 mL). The combined aqueous phases were extracted with dichloromethane (3 × 150 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (16.7% ethyl acetate-hexane) afforded pyrido[3,2-c]coumarin (85 mg, 85%) as a white solid; R_f 0.39 (20% ethyl acetate-hexane); mp 165-166 °C; ν_{max} (solid) 3074, 1724 (C=O), 1604, 1448, 1260, 1087, 755, 728 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 9.03 (dd, 1H, J 4.7, 1.8 Hz, H-2), 8.65-8.59 (m, 2H, H-10, H-8), 7.62-7.56 (m, 1H, H-7), 7.53 (dd, 1H, J 8.0, 4.7 Hz, H-3), 7.62-7.57 (m, 2H, H-4, H-9); δ_c (100 MHz, $CDCl_3$) 161.3 (C=O), 155.9 (C-2), 152.8 (C-10b), 152.1 (C-6a), 138.4 (C-4), 132.4 (C-8), 125.1 (C-10), 124.9 (C-9), 123.9 (C-3), 119.5 (C-10a), 117.6 (C-4a), 117.4 (C-7). *In agreement with published data*.[135]

Microwave-assisted reaction conditions:

A mixture of 2-chloronicotinonitrile (50 mg, 0.36 mmol, 1.0 equiv.),

2-hydroxyphenylboronic acid (59 mg, 0.43 mmol, 1.2 equiv.), tetrakis(triphenylphosphine)palladium(0) (42 mg, 0.04 mmol, 0.1 equiv.) was degassed with N_2. 1,4-Dioxane (3.6 mL), water (0.36 mL) and potassium carbonate solution (2 M aqueous solution; 0.72 mL, 1.44 mmol, 4.0 equiv.) were added at rt, and the resulting mixture stirred in the microwave reactor at 125 ºC for 30 min (power 300 W). The mixture was diluted with dichloromethane (150 mL) and water (20 mL). The layers were separated and the organic phase washed with brine (20 mL). The combined aqueous phases were extracted with dichloromethane (3 × 150 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (16.7% ethyl acetate-hexane) afforded pyrido[3,2-*c*]coumarin (56 mg, 79%) as a white solid.

9-Chloropyrido[3,2-*c*]coumarin



Thermal reaction conditions:

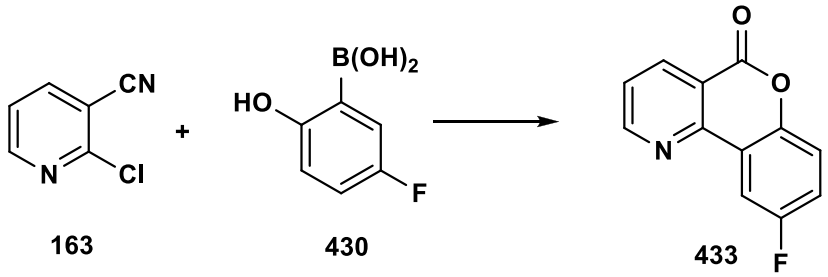
A mixture of 2-chloronicotinonitrile (50 mg, 0.36 mmol, 1.0 equiv.), 5-chloro-2-hydroxyphenylboronic acid (75 mg, 0.43 mmol, 1.2 equiv.), tetrakis(triphenylphosphine)palladium(0) (42 mg, 0.04 mmol, 0.1 equiv.) and 1,4-dioxane (3.6 mL) was stirred at 80 ºC for 1 h, then water (0.36 mL) and potassium carbonate solution (2 M aqueous solution; 0.72 mL, 1.44 mmol, 4.0 equiv.) were added at rt and the resulting mixture stirred at 80 ºC for 12 h. The mixture was diluted with dichloromethane (150 mL) and water (20 mL). The layers were separated and the organic phase washed with brine (20 mL). The combined aqueous phases were extracted

with dichloromethane (3 × 150 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (14.3% ethyl acetate-hexane) afforded 9-chloropyrido[3,2-*c*]coumarin (63 mg, 75%) as a white solid; R_f (25% ethyl acetate-hexane); mp 189-190 °C; ν_{max} (solid) 3075, 1732 (C=O), 1563, 1445, 1394, 1254, 1096, 1070, 1038, 825, 788, 723 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 9.04 (dd, 1H, J 4.7, 1.7 Hz, H-2), 8.63 (dd, 1H, J 8.0, 1.7 Hz, H-4), 8.59 (d, 1H, J 2.6 Hz, H-10), 7.59-7.52 (m, 2H, H-8, H-3), 7.34 (d, 1H, J 8.8 Hz, H-7); δ_c (100 MHz, $CDCl_3$) 160.8 (C=O), 156.0 (C-2), 151.1 (C-10b), 151.0 (C-6a), 138.5 (C-4), 132.2 (C-8), 130.8 (C-9), 124.6 (C-10), 124.5 (C-3), 120.7 (C-10a), 118.9 (C-7), 117.7 (C-4a); *m/z* (ESI) 232 $[M+H]^+$ (Found: $[M+H]^+$, 232.0159. $C_{12}H_6NO_2Cl$ requires $[M+H]^+$, 232.0160).

Microwave-assisted reaction conditions:

A mixture of 2-chloronicotinonitrile (50 mg, 0.36 mmol, 1.0 equiv.), 5-chloro-2-hydroxyphenylboronic acid (75 mg, 0.43 mmol, 1.2 equiv.), tetrakis(triphenylphosphine)palladium(0) (42 mg, 0.04 mmol, 0.1 equiv.) was degassed with N_2. 1,4-Dioxane (3.6 mL), water (0.36 mL) and potassium carbonate solution (2 M aqueous solution; 0.72 mL, 1.44 mmol, 4.0 equiv.) were added at rt, and the resulting mixture stirred in the microwave reactor at 125 ºC for 30 min (power 300 W). The mixture was diluted with dichloromethane (150 mL) and water (20 mL). The layers were separated and the organic phase washed with brine (20 mL). The combined aqueous phases were extracted with dichloromethane (3 × 150 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (14.3% ethyl acetate-hexane) afforded 9-chloropyrido[3,2-*c*]coumarin (63 mg, 76%) as a white solid.

9-Fluoropyrido[3,2-*c*]coumarin



163 430 433

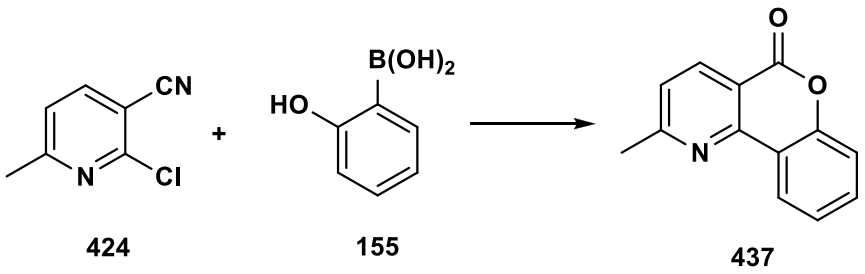
Thermal reaction conditions:

A mixture of 2-chloronicotinonitrile (50 mg, 0.36 mmol, 1.0 equiv.), 5-fluoro-2-hydroxyphenylboronic acid (68 mg, 0.43 mmol, 1.2 equiv.), tetrakis(triphenylphosphine)palladium(0) (42 mg, 0.04 mmol, 0.1 equiv.) and 1,4-dioxane (3.6 mL) was stirred at 80 ºC for 1 h, then water (0.36 mL) and potassium carbonate solution (2 M aqueous solution; 0.72 mL, 1.44 mmol, 4.0 equiv.) were added at rt and the resulting mixture stirred at 80 ºC for 12 h. The mixture was diluted with dichloromethane (150 mL) and water (20 mL). The layers were separated and the organic phase washed with brine (20 mL). The combined aqueous phases were extracted with dichloromethane (3 × 150 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (18.2% ethyl acetate-hexane) afforded 9-fluoropyrido[3,2-*c*]coumarin (60 mg, 77%) as a white solid; R_f 0.51 (25% ethyl acetate-petrol); mp 187-188 °C; ν_{max} (solid) 3082, 1742 (C=O), 1570, 1470, 1450, 1260, 1176, 1129, 1097, 1040, 882, 818, 786 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 9.04 (dd, 1H, J 4.7, 1.8 Hz, H-2), 8.64 (dd, 1H, J 8.0, 1.8 Hz, H-4), 8.26 (dd, 1H, J 8.7, 3.0 Hz, H-10), 7.57 (dd, 1H, J 8.0, 4.7 Hz, H-3), 7.38 (dd, 1H, J 9.0, 4.4 Hz, H-7), 7.29 (ddd, 1H, J 9.0, 7.0, 3.0 Hz, H-8); δ_c (100 MHz, $CDCl_3$) 160.9 (C=O), 159.7 (d, $^1J_{CF}$ 244.3 Hz, C-9), 155.9 (C-2), 151.3 (d, $^4J_{CF}$ 2.8 Hz, C-10b), 148.8 (d, $^4J_{CF}$ 2.2 Hz, C-6a), 138.4 (C-4), 124.5 (C-3), 120.8 (d, $^3J_{CF}$ 8.7 Hz, C-10a), 119.7 (d, $^2J_{CF}$ 24.7 Hz, C-8), 119.0 (d, $^3J_{CF}$ 8.3 Hz, C-7), 117.7 (C-4a), 110.7 ($^2J_{CF}$ 25.4 Hz, C-10); δ_F (376 MHz, $CDCl_3$) -116.4 (s); *m/z* (ESI) 216 $[M+H]^+$ (Found: $[M+H]^+$, 216.0455. $C_{12}H_6NO_2F$ requires $[M+H]^+$, 216.0455). *In agreement with published data*.[136]

Microwave-assisted reaction conditions:

A mixture of 2-chloronicotinonitrile (50 mg, 0.36 mmol, 1.0 equiv.), 5-fluoro-2-hydroxyphenylboronic acid (68 mg, 0.43 mmol, 1.2 equiv.), tetrakis(triphenylphosphine)palladium(0) (42 mg, 0.04 mmol, 0.1 equiv.) was degassed with N_2. 1,4-Dioxane (3.6 mL), water (0.36 mL) and potassium carbonate solution (2 M aqueous solution; 0.72 mL, 1.44 mmol, 4.0 equiv.) were added at rt, and the resulting mixture stirred in the microwave reactor at 125 ºC for 30 min (power 300 W). The mixture was diluted with dichloromethane (150 mL) and water (20 mL). The layers were separated and the organic phase washed with brine (20 mL). The combined aqueous phases were extracted with dichloromethane (3 × 150 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (18.2% ethyl acetate-hexane) afforded 9-fluoropyrido[3,2-*c*]coumarin (52 mg, 66%) as a white solid.

2-Methylpyrido[3,2-*c*]coumarin



424 155 437

Thermal reaction conditions:

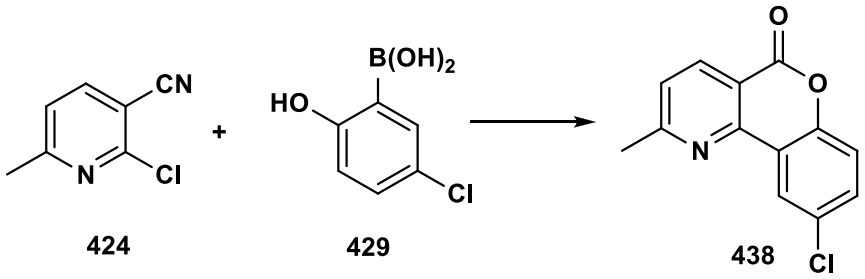
A mixture of 2-chloro-6-methylnicotinonitrile (50 mg, 0.33 mmol, 1.0 equiv.), 2-hydroxyphenylboronic acid (54 mg, 0.39 mmol, 1.2 equiv.), tetrakis(triphenylphosphine)palladium(0) (38 mg, 0.03 mmol, 0.1 equiv.) and 1,4-dioxane (3.3 mL) was stirred at 80 ºC for 1 h, then water (0.33 mL) and potassium carbonate solution (2 M aqueous solution; 0.66 mL, 1.32 mmol, 4.0 equiv.) were added at rt and the resulting mixture stirred at 80 ºC for 12 h. The mixture was diluted with dichloromethane (150 mL) and water (20 mL). The layers were separated and the

organic phase washed with brine (20 mL). The combined aqueous phases were extracted with dichloromethane (3 × 150 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (11.1% ethyl acetate-hexane) afforded 2-methylpyrido[3,2-*c*]coumarin (54 mg, 78%) as a white solid; R_f 0.67 (43.5% ethyl acetate-petrol); mp 162-163 °C; ν_{max} (solid) 2923, 1723 (C=O), 1569, 1430, 1268, 1099, 755, 735 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.60 (dd, 1H, J 7.8, 1.3 Hz, H-10), 8.47 (d, 1H, J 8.1 Hz, H-4), 7.60-7.50 (m, 1H, H-8), 7.41-7.31 (m, 3H, H-7, H-9, H-3), 2.76 (s, 3H, 2-C<u>H</u>$_3$); δ_c (100 MHz, $CDCl_3$) 166.2 (C-2), 161.5 (C=O), 152.8 (C-10b), 151.5 (C-6a), 138.2 (C-4), 132.1 (C-8), 124.9 (C-10), 124.8 (C-9), 124.0 (C-3), 119.5 (C-10a), 117.3 (C-7), 115.0 (C-4a), 25.7 (2-<u>C</u>H_3); *m/z* (ESI) 212 $[M+H]^+$ (Found: $[M+H]^+$, 212.0705. $C_{13}H_9NO_2$ requires $[M+H]^+$, 212.0712).

Microwave-assisted reaction conditions:

A mixture of 2-chloro-6-methylnicotinonitrile (50 mg, 0.33 mmol, 1.0 equiv.), 2-hydroxyphenylboronic acid (54 mg, 0.39 mmol, 1.2 equiv.), tetrakis(triphenylphosphine)palladium(0) (38 mg, 0.03 mmol, 0.1 equiv.) was degassed with N_2. 1,4-Dioxane (3.3 mL), water (0.36 mL) and potassium carbonate solution (2 M aqueous solution; 0.66 mL, 1.32 mmol, 4.0 equiv.) were added at rt, and the resulting mixture stirred in the microwave reactor at 125 ºC for 30 min (power 300 W). The mixture was diluted with dichloromethane (150 mL) and water (20 mL). The layers were separated and the organic phase washed with brine (20 mL). The combined aqueous phases were extracted with dichloromethane (3 × 150 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (11.1% ethyl acetate-hexane) afforded 2-methylpyrido[3,2-*c*]coumarin (51 mg, 74%) as a white solid.

9-Chloro-2-methylpyrido[3,2-*c*]coumarin



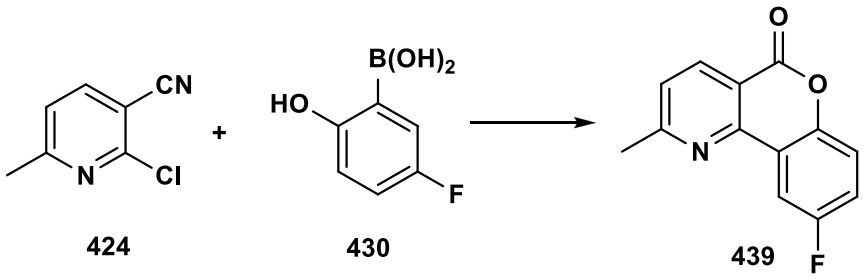
Thermal reaction conditions:

A mixture of 2-chloro-6-methylnicotinonitrile (50 mg, 0.33 mmol, 1.0 equiv.), 5-chloro-2-hydroxyphenylboronic acid (68 mg, 0.39 mmol, 1.2 equiv.), tetrakis(triphenylphosphine)palladium(0) (38 mg, 0.03 mmol, 0.1 equiv.) and 1,4-dioxane (3.3 mL) was stirred at 80 ºC for 1 h, then water (0.33 mL) and potassium carbonate solution (2 M aqueous solution; 0.66 mL, 1.32 mmol, 4.0 equiv.) were added at rt and the resulting mixture stirred at 80 ºC for 12 h. The mixture was diluted with dichloromethane (150 mL) and water (20 mL). The layers were separated and the organic phase washed with brine (20 mL). The combined aqueous phases were extracted with dichloromethane (3 × 150 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (11.1% ethyl acetate-hexane) afforded 9-chloro-2-methylpyrido[3,2-*c*]coumarin (58 mg, 71%) as a white solid; R_f 0.64 (33.3% ethyl acetate-hexane); mp 192-193 °C; ν_{max} (solid) 3068, 1757 (C=O), 1568, 1386, 1249, 1103, 1041, 821, 792 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.59 (d, 1H, J 2.6 Hz, H-10), 8.48 (d, 1H, J 8.1 Hz, H-4), 7.51 (dd, 1H, J 8.8, 2.6 Hz, H-8), 7.41 (d, 1H, J 8.1 Hz, H-3), 7.32 (d, 1H, J 8.8 Hz, H-7), 2.77 (s, 3H, 2-C$\underline{H}_3$); δ_c (100 MHz, $CDCl_3$) 166.5 (C-2), 161.0 (C=O), 151.2 (C-10b), 150.5 (C-6a), 138.3 (C-4), 132.0 (C-8), 130.6 (C-9), 124.6 (C-10), 124.6 (C-3), 120.9 (C-10a), 118.8 (C-7), 115.2 (C-4a), 25.7 (2-$\underline{C}H_3$); δ_F (376 MHz, $CDCl_3$) –116.8 (s); *m/z* (ESI) 246 $[M+H]^+$ (Found: $[M+H]^+$, 246.0317. $C_{13}H_8NO_2Cl$ requires $[M+H]^+$, 246.0316).

Microwave-assisted reaction conditions:

A mixture of 2-chloro-6-methylnicotinonitrile (50 mg, 0.33 mmol, 1.0 equiv.), 5-chloro-2-hydroxyphenylboronic acid (68 mg, 0.39 mmol, 1.2 equiv.), tetrakis(triphenylphosphine)palladium(0) (38 mg, 0.03 mmol, 0.1 equiv.) was degassed with N_2. 1,4-Dioxane (3.3 mL), water (0.33 mL) and potassium carbonate solution (2 M aqueous solution; 0.66 mL, 1.32 mmol, 4.0 equiv.) were added at rt, and the resulting mixture stirred in the microwave reactor at 125 ºC for 30 min (power 300 W). The mixture was diluted with dichloromethane (150 mL) and water (20 mL). The layers were separated and the organic phase washed with brine (20 mL). The combined aqueous phases were extracted with dichloromethane (3 × 150 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (11.1% ethyl acetate-hexane) afforded 9-chloro-2-methylpyrido[3,2-*c*]coumarin (51 mg, 63%) as a white solid.

9-Fluoro-2-methylpyrido[3,2-*c*]coumarin



424 430 439

Thermal reaction conditions:

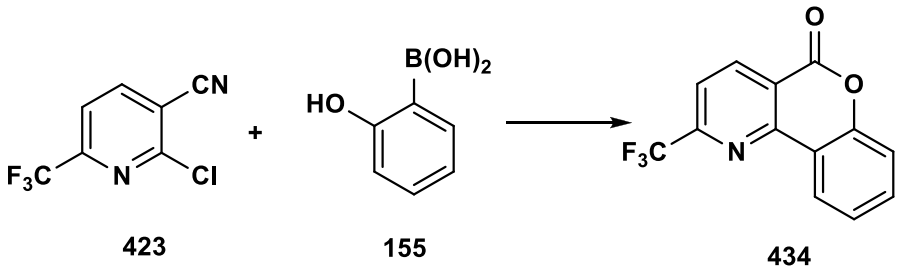
A mixture of 2-chloro-6-methylnicotinonitrile (53 mg, 0.35mmol, 1.0 equiv.), 5-fluoro-2-hydroxyphenylboronic acid (65 mg, 0.42 mmol, 1.2 equiv.), tetrakis(triphenylphosphine)palladium(0) (40 mg, 0.04 mmol, 0.1 equiv.) and 1,4-dioxane (3.5 mL) was stirred at 80 ºC for 1 h, then water (0.35 mL) and potassium carbonate solution (2 M aqueous solution; 0.70 mL, 1.40 mmol, 4.0 equiv.) were added at rt and the resulting mixture stirred at 80 ºC for 12 h. The mixture was diluted with dichloromethane (150 mL) and water (20 mL). The layers were separated and the

organic phase washed with brine (20 mL). The combined aqueous phases were extracted with dichloromethane (3 × 150 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (13.3% ethyl acetate-hexane) afforded 9-fluoro-2-methylpyrido[3,2-*c*]coumarin (56 mg, 70%) as a white solid; R_f 0.74 (38.5% ethyl acetate-hexane); mp 186-187 °C; ν_{max} (solid) 3078, 1717 (C=O), 1572, 1473, 1430, 1246, 1172, 1125, 1071, 1044, 884, 830, 793 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.49 (d, 1H, J 8.1, H-4), 8.29 (dd, 1H, J 8.7, 3.1 Hz, H-10), 7.41 (d, 1H, J 8.1 Hz, H-3), 7.36 (dd, 1H, J 9.0, 4.4 Hz, H-7), 7.30-7.27 (m, 1H, H-8), 2.77 (s, 3H, 2-C$\underline{H}_3$); δ_c (100 MHz, $CDCl_3$) 166.3 (C-2), 161.2 (C=O), 159.6 (d, $^1J_{CF}$ 243.9 Hz, C-9), 150.8 (d, $^4J_{CF}$ 2.8 Hz, C-10b), 149.0 (d, $^4J_{CF}$ 2.1 Hz, C-6a), 138.3 (C-4), 124.6 (C-3), 120.9 (d, $^3J_{CF}$ 8.7 Hz, C-10a), 119.4 (d, $^2J_{CF}$ 24.7 Hz, C-8), 118.9 (d, $^3J_{CF}$ 8.3 Hz, C-7), 115.1 (C-4a), 110.7 ($^2J_{CF}$ 25.3 Hz, C-10), 25.6 (2-$\underline{C}H_3$); δ_F (376 MHz, $CDCl_3$) – 116.8 (s); *m/z* (ESI) 230 $[M+H]^+$ (Found: $[M+H]^+$, 230.0610. $C_{13}H_8NO_2F$ requires $[M+H]^+$, 230.0612).

Microwave-assisted reaction conditions:

A mixture of 2-chloro-6-methylnicotinonitrile (50 mg, 0.33 mmol, 1.0 equiv.), 5-fluoro-2-hydroxyphenylboronic acid (61 mg, 0.39 mmol, 1.2 equiv.), tetrakis(triphenylphosphine)palladium(0) (38 mg, 0.03 mmol, 0.1 equiv.) was degassed with N_2. 1,4-Dioxane (3.3 mL), water (0.33 mL) and potassium carbonate solution (2 M aqueous solution; 0.66 mL, 1.32 mmol, 4.0 equiv.) were added at rt, and the resulting mixture stirred in the microwave reactor at 125 ºC for 30 min (power 300 W). The mixture was diluted with dichloromethane (150 mL) and water (20 mL). The layers were separated and the organic phase washed with brine (20 mL). The combined aqueous phases were extracted with dichloromethane (3 × 150 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (13.3% ethyl acetate-hexane) afforded 9-fluoro-2-methylpyrido[3,2-*c*]coumarin (53 mg, 69%) as a white solid.

2-(Trifluoromethyl)pyrido[3,2-*c*]coumarin



423 155 434

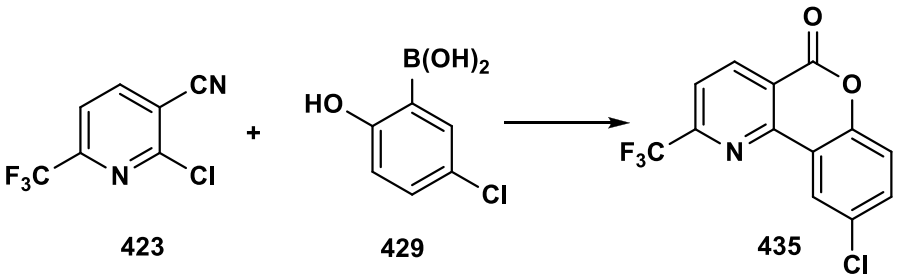
Thermal reaction conditions:

A mixture of 2-chloro-6-(trifluoromethyl)nicotinonitrile (52 mg, 0.25 mmol, 1.0 equiv.), 2-hydroxyphenylboronic acid (42 mg, 0.30 mmol, 1.2 equiv.), tetrakis(triphenylphosphine)palladium(0) (29 mg, 0.03 mmol, 0.1 equiv.) and 1,4-dioxane (2.5 mL) was stirred at 80 ºC for 1 h, then water (0.25 mL) and potassium carbonate solution (2 M aqueous solution; 0.50 mL, 1.00 mmol, 4.0 equiv.) were added at rt and the resulting mixture stirred at 80 ºC for 12 h. The mixture was diluted with dichloromethane (150 mL) and water (20 mL). The layers were separated and the organic phase washed with brine (20 mL). The combined aqueous phases were extracted with dichloromethane (3 × 150 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (11.1% ethyl acetate-hexane) afforded 2-trifluoromethylpyrido[3,2-*c*]coumarin (60 mg, 90%) as a white solid; R_f 0.76 (20% ethyl acetate-hexane); mp 176-178°C; ν_{max} (solid) 3094, 1744 (C=O), 1346, 1260, 1190, 1147, 1131, 1113, 1103, 861, 762, 742, 720 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.82 (d, 1H, J 8.2 Hz, H-4), 8.66 (dd, 1H, J 7.9, 1.6 Hz, H-10), 7.87 (d, 1H, J 8.2 Hz, H-3), 7.67-7.83 (m, 1H, H-8), 7.47-7.41 (m, 2H, H-7, H-9); δ_c (100 MHz, $CDCl_3$) 160.1 (C=O), 153.5 (q, $^2J_{CF}$ 35.6 Hz, C-2), 153.0 (C-10b), 152.4 (C-6a), 140.7 (C-4), 133.4 (C-8), 125.5 (C-10), 125.4 (C-9), 121.0 (q, $^1J_{CF}$ 275.5 Hz, $\underline{C}F_3$), 120.0 (q, $^3J_{CF}$ 2.5 Hz, C-3), 119.4 (C-10a), 118.5 (C-4a), 117.5 (C-7); δ_F (376 MHz, $CDCl_3$) -68.5 (s); *m/z* (ESI) 266 $[M+H]^+$ (Found: $[M+H]^+$, 266.0422. $C_{13}H_6NO_2F_3$ requires $[M+H]^+$, 266.0423).

Microwave-assisted reaction conditions:

A mixture of 2-chloro-6-(trifluoromethyl)nicotinonitrile (50 mg, 0.24 mmol, 1.0 equiv.), 2-hydroxyphenylboronic acid (40 mg, 0.29 mmol, 1.2 equiv.), tetrakis(triphenylphosphine)palladium(0) (28 mg, 0.02 mmol, 0.1 equiv.) was degassed with N_2. 1,4-Dioxane (2.4 mL), water (0.24 mL) and potassium carbonate solution (2 M aqueous solution; 0.48 mL, 0.96 mmol, 4.0 equiv.) were added at rt, and the resulting mixture stirred in the microwave reactor at 125 ºC for 30 min (power 300 W). The mixture was diluted with dichloromethane (150 mL) and water (20 mL). The layers were separated and the organic phase washed with brine (20 mL). The combined aqueous phases were extracted with dichloromethane (3 × 150 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (11.1% ethyl acetate-hexane) afforded 2-trifluoromethylpyrido[3,2-*c*]coumarin (52 mg, 80%) as a white solid.

9-Chloro-2-(trifluoromethyl)pyrido[3,2-*c*]coumarin



423 429 435

Thermal reaction conditions:

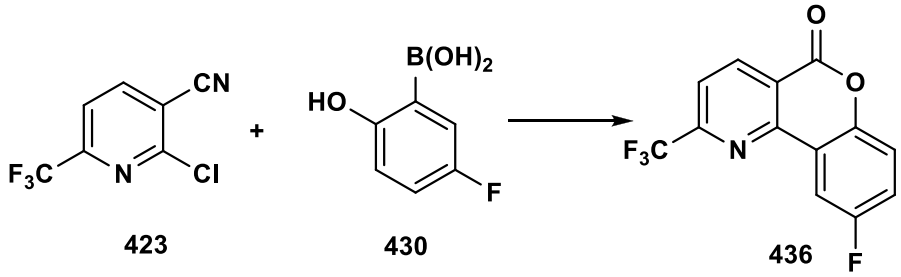
A mixture of 2-chloro-6-(trifluoromethyl)nicotinonitrile (55 mg, 0.26 mmol, 1.0 equiv.), 5-chloro-2-hydroxyphenylboronic acid (55 mg, 0.32 mmol, 1.2 equiv.), tetrakis(triphenylphosphine)palladium(0) (31 mg, 0.03 mmol, 0.1 equiv.) and 1,4-dioxane (2.6 mL) was stirred at 80 ºC for 1 h, then water (0.26 mL) and potassium carbonate solution (2 M aqueous solution; 0.52 mL,1.04 mmol, 4.0 equiv.) were added at rt and the resulting mixture stirred at 80 ºC for 12 h. The mixture was diluted with

dichloromethane (150 mL) and water (20 mL). The layers were separated and the organic phase washed with brine (20 mL). The combined aqueous phases were extracted with dichloromethane (3 × 150 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (11.8% ethyl acetate-hexane) afforded 9-chloro-2-(trifluoromethyl)pyrido[3,2-*c*]coumarin (64 mg, 81%) as a white solid; R_f 0.83 (25% ethyl acetate-hexane); mp 193-194 °C; ν_{max} (solid) 3107, 1743 (C=O), 1339, 1261, 1177, 1157, 1106, 1088, 864, 828, 724 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.83 (d, 1H, J 8.2 Hz, H-4), 8.60 (d, 1H, J 2.6 Hz, H-10), 7.91 (d, 1H, J 8.2 Hz, H-3), 7.59 (dd, 1H, J 8.8, 2.6 Hz, H-8), 7.37 (d, 1H, J 8.8 Hz, H-7); δ_c (100 MHz, $CDCl_3$) 159.5 (C=O), 153.7 (q, $^2J_{CF}$ Hz, C-2), 151.4 (C-10b), 151.3 (C-6a), 140.9 (C-4), 133.4 (C-8), 131.2 (C-9), 125.0 (C-10), 120.9 (q, $^1J_{CF}$ 275.7 Hz, $\underline{C}F_3$), 120.7 (q, $^3J_{CF}$ 2.4 Hz, C-3), 119.7 (C-10a), 119.6 (C-4a), 119.0 (C-7); δ_F (376 MHz, $CDCl_3$) -68.5 (s). *m/z* (ESI) 300 $[M+H]^+$ (Found: $[M+H]^+$, 300.0033. $C_{13}H_5NO_2F_3Cl$ requires $[M+H]^+$, 300.0034).

Microwave-assisted reaction conditions:

A mixture of 2-chloro-6-(trifluoromethyl)nicotinonitrile (68.3 mg, 0.33 mmol, 1.0 equiv.), 5-chloro-2-hydroxyphenylboronic acid (68.4 mg, 0.40 mmol, 1.2 equiv.), tetrakis(triphenylphosphine)palladium(0) (38.2 mg, 0.03 mmol, 0.1 equiv.) was degassed with N_2. 1,4-Dioxane (3.3 mL), water (0.33 mL) and potassium carbonate solution (2 M aqueous solution; 0.66 mL, 1.32 mmol, 4.0 equiv.) were added at rt, and the resulting mixture stirred in the microwave reactor at 125 ºC for 30 min (power 300 W). The mixture was diluted with dichloromethane (150 mL) and water (20 mL). The layers were separated and the organic phase washed with brine (20 mL). The combined aqueous phases were extracted with dichloromethane (3 × 150 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (11.8% ethyl acetate-hexane) afforded 9-chloro-2-(trifluoromethyl)pyrido[3,2-*c*]coumarin (74.5 mg, 75%) as a white solid.

9-Fluoro-2-(trifluoromethyl)pyrido[3,2-*c*]coumarin



423 430 436

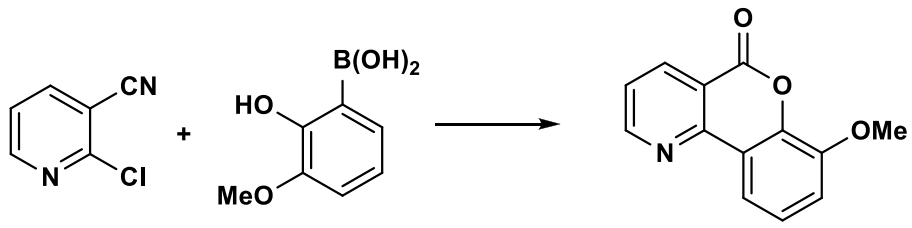
Thermal reaction conditions:

A mixture of 2-chloro-6-(trifluoromethyl)nicotinonitrile (58 mg, 0.28 mmol, 1.0 equiv.), 5-fluoro-2-hydroxyphenylboronic acid (52 mg, 0.34 mmol, 1.2 equiv.), tetrakis(triphenylphosphine)palladium(0) (32 mg, 0.03 mmol, 0.1 equiv.) and 1,4-dioxane (2.8 mL) was stirred at 80 ºC for 1 h, then water (0.28 mL) and potassium carbonate solution (2 M aqueous solution; 0.56 mL, 1.12 mmol, 4.0 equiv.) were added at rt and the resulting mixture stirred at 80 ºC for 12 h. The mixture was diluted with dichloromethane (150 mL) and water (20 mL). The layers were separated and the organic phase washed with brine (20 mL). The combined aqueous phases were extracted with dichloromethane (3 × 150 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (11.8% ethyl acetate-hexane) afforded 9-fluoro-2-(trifluoromethyl)pyrido[3,2-*c*]coumarin (62 mg, 78%) as a white solid; R_f 0.82 (25% ethyl acetate-hexane); mp 150-151 °C; ν_{max} (solid) 3102, 1742 (C=O), 1440, 1342, 1261, 1156, 1113, 1098, 1038, 887, 834, 808, 723; δ_H (400 MHz, $CDCl_3$) 8.83 (d, 1H, J 8.2 Hz, H-4), 8.30 (dd, 1H, J 8.4, 3.0 Hz, H-10), 7.90 (d, 1H, J 8.2 Hz, H-3), 7.41 (dd, 1H, J 9.0, 4.4 Hz, H-7), 7.35 (ddd, 1H, J 9.0, 7.5, 3.0 Hz, H-8); δ_c (100 MHz, $CDCl_3$) 159.8 (q, $^2J_{CF}$ 245.5 Hz, C-2), 159.7 (C=O), 153.6 (q, $^2J_{CF}$ 35.7 Hz, C-2), 151.6 (d, $^4J_{CF}$ 2.7 Hz, C-6a), 149.1 (d, $^4J_{CF}$ 2.2 Hz, C-10b), 140.9 (C-4), 120.9 (q, $^1J_{CF}$ 275.5 Hz, $\underline{C}F_3$), 120.86 (d, $^2J_{CF}$ 24.8 Hz, C-8), 120.7 (q, $^3J_{CF}$ 2.4 Hz, C-3), 119.8 (d, $^3J_{CF}$ 8.8 Hz, C-10a), 119.5 (C-4a), 119.2 (d, $^3J_{CF}$ 8.3 Hz, C-7), 111.2 ($^2J_{CF}$ 25.5 Hz, C-10); δ_F (376 MHz, $CDCl_3$) -68.3 (s), -115.5 (s); *m/z* (ESI) 284 $[M+H]^+$ (Found: $[M+H]^+$, 284.0329. $C_{13}H_5NO_2F_4$ requires $[M+H]^+$, 284.0329).

Microwave-assisted reaction conditions:

A mixture of 2-chloro-6-(trifluoromethyl)nicotinonitrile (70 mg, 0.34 mmol, 1.0 equiv.), 5-fluoro-2-hydroxyphenylboronic acid (70 mg, 0.41 mmol, 1.2 equiv.), tetrakis(triphenylphosphine)palladium(0) (39.1 mg, 0.03 mmol, 0.1 equiv.) was degassed with N_2. 1,4-Dioxane (3.4 mL), water (0.34 mL) and potassium carbonate solution (2 M aqueous solution; 0.68 mL, 1.36 mmol, 4.0 equiv.) were added at rt, and the resulting mixture stirred in the microwave reactor at 125 ºC for 30 min (power 300 W). The mixture was diluted with dichloromethane (150 mL) and water (20 mL). The layers were separated and the organic phase washed with brine (20 mL). The combined aqueous phases were extracted with dichloromethane (3 × 150 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (11.8% ethyl acetate-hexane) afforded 9-fluoro-2-(trifluoromethyl)pyrido[3,2-*c*]coumarin (69 mg, 72%) as a white solid.

7-Methoxypyrido[3,2-*c*]coumarin

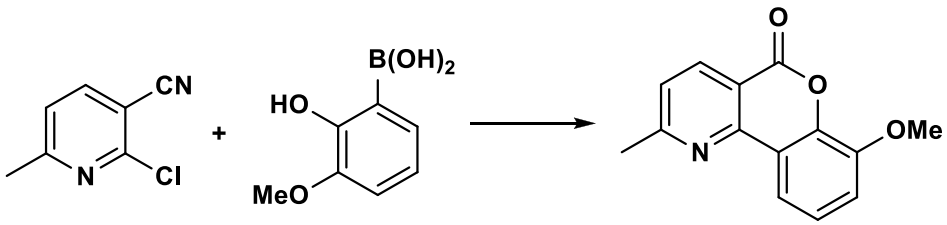


163 431 440

A mixture of 2-chloronicotinonitrile (51 mg, 0.37 mmol, 1.0 equiv.), 2-hydroxy-3-methoxyphenylboronic acid (75 mg, 0.44 mmol, 1.2 equiv.), tetrakis(triphenylphosphine)palladium(0) (43 mg, 0.04 mmol, 0.1 equiv.) and 1,4-dioxane (3.7 mL) was stirred at 80 ºC for 1 h, then water (0.37 mL) and potassium carbonate solution (2 M aqueous solution; 0.74 mL, 1.48 mmol, 4.0 equiv.) were added at rt and the resulting mixture stirred at 80 ºC for 12 h. The mixture was diluted with dichloromethane (150 mL) and water (20 mL). The layers were separated and the organic phase washed with brine (20 mL). The combined aqueous phases were extracted

with dichloromethane (3 × 150 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (20% ethyl acetate-hexane) afforded 7-methoxypyrido[3,2-*c*]coumarin (64 mg, 76%) as a white solid; R_f 0.34 (20% ethyl acetate-hexane); mp 215-217 °C; ν_{max} (solid) 2918, 1721 (C=O), 1436, 1388, 1269, 1188, 1093, 1029, 754, 723, 691 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 9.03 (dd, 1H, J 4.6, 1.6 Hz, H-2), 8.64 (dd, 1H, J 8.0, 1.6 Hz, H-4), 8.18 (dd, 1H, J 8.0, 1.2 Hz, H-10), 7.53 (dd, 1H, J 8.0, 4.6 Hz, H-3), 7.34 (t, 1H, J 8.0 Hz, H-9), 7.15 (dd, 1H, J 8.0, 1.2 Hz, H-8), 4.01 (s, 3H, O-C<u>H</u>$_3$); δ_c (100 MHz, $CDCl_3$) 160.8 (C=O), 155.8 (C-10b), 152.3 (C-2), 147.8 (C-6a), 142.6 (C-7), 138.4 (C-4), 124.8 (C-9), 124.0 (C-10a), 120.4 (C-4a), 117.7 (C-3), 116.2 (C-10), 114.3 (C-8), 56.5 (O-<u>C</u>H_3); *m/z* (ESI) 228 $[M+H]^+$ (Found: $[M+H]^+$, 228.0651. $C_{13}H_9NO_3$ requires $[M+H]^+$, 228.0661).

7-Methoxy-2-methylpyrido[3,2-*c*]coumarin

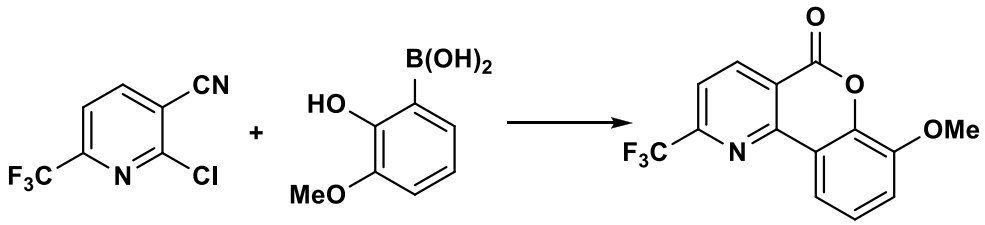


4 431 442

A mixture of 2-chloro-6-methylnicotinonitrile (51 mg, 0.33 mmol, 1.0 equiv.), 2-hydroxy-3-methoxyphenylboronic acid (67 mg, 0.40 mmol, 1.2 equiv.), tetrakis(triphenylphosphine)palladium(0) (38 mg, 0.03 mmol, 0.1 equiv.) and 1,4-dioxane (3.3 mL) was stirred at 80 ºC for 1 h, then water (0.33 mL) and potassium carbonate solution (2 M aqueous solution; 0.66 mL, 1.32 mmol, 4.0 equiv.) were added at rt and the resulting mixture stirred at 80 ºC for 12 h. The mixture was diluted with dichloromethane (150 mL) and water (20 mL). The layers were separated and the organic phase washed with brine (20 mL). The combined aqueous phases were extracted with dichloromethane (3 × 150 mL) and the combined organic phases dried (Na_2SO_4).

Concentration under reduced pressure and chromatography (15.4% ethyl acetate-hexane) afforded 7-methoxy-2-methylpyrido[3,2-*c*]coumarin (58 mg, 72%) as a white solid; R_f 0.58 (0.7% methanol-dichloromethane); mp 210-212 °C; ν_{max} (film) 2915, 1721 (C=O), 1582, 1433, 1272, 1134, 1085, 1067, 766, 710; δ_H (400 MHz, $CDCl_3$) 8.49 (d, 1H, J 8.1 Hz, H-4), 8.19 (dd, 1H, J 8.0, 1.3 Hz, H-10), 7.36 (d, 1H, J 8.1 Hz, H-3), 7.31 (t, 1H, J 8.0 Hz, H-9), 7.12 (dd, 1H, J 8.0, 1.3 Hz, H-8), 4.00 (s, 3H, O-C$\underline{H}_3$), 2.76 (s, 3H, C$\underline{H}_3$); δ_c (100 MHz, $CDCl_3$) 166.1 (C-2), 161.0 (C=O), 151.7 (C-10b), 147.8 (C-6a), 142.7 (C-7), 138.3 (C-4), 124.5 (C-9), 124.1 (C-10a), 120.5 (C-4a), 116.2 (C-3), 115.1 (C-10), 114.0 (C-8), 56.5 (O-$\underline{C}H_3$), 25.7 (2-$\underline{C}H_3$); *m/z* (ESI) 242 $[M+H]^+$ (Found: $[M+H]^+$, 242.0809. $C_{14}H_{11}NO_3$ requires $[M+H]^+$, 242.0817).

7-Methoxy-2-(trifluoromethyl)pyrido[3,2-*c*]coumarin

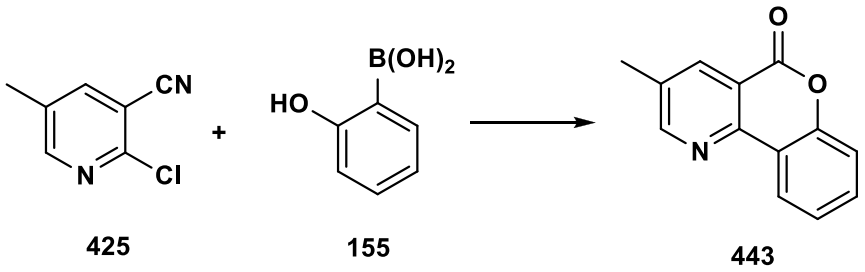


423 431 441

A mixture of 2-chloro-6-(trifluoromethyl)nicotinonitrile (57 mg, 0.27 mmol, 1.0 equiv.), 2-hydroxy-3-methoxyphenylboronic acid (55 mg, 0.33 mmol, 1.2 equiv.), tetrakis(triphenylphosphine)palladium(0) (32 mg, 0.03 mmol, 0.1 equiv.) and 1,4-dioxane (2.7 mL) was stirred at 80 ºC for 1 h, then water (0.27 mL) and potassium carbonate solution (2 M aqueous solution; 0.54 mL, 1.08 mmol, 4.0 equiv.) were added at rt and the resulting mixture stirred at 80 ºC for 12 h. The mixture was diluted with dichloromethane (150 mL) and water (20 mL). The layers were separated and the organic phase washed with brine (20 mL). The combined aqueous phases were extracted with dichloromethane (3 × 150 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (13.3% ethyl acetate-hexane) afforded 7-methoxy-2-(trifluoromethyl)pyrido[3,2-*c*]coumarin (65 mg, 80%) as a white

solid; R_f 0.71 (37% ethyl acetate-hexane); mp 180-182 °C; ν_{max} (solid) 3085, 1745 (C=O), 1586, 1412, 1263, 1179, 1133, 1112, 1053, 782, 732; δ_H (400 MHz, $CDCl_3$) 8.82 (d, 1H, J 8.2 Hz, H-4), 8.20 (dd, 1H, J 8.0, 1.2 Hz, H-10), 7.86 (d, 1H, J 8.2 Hz, H-3), 7.36 (t, 1H, J 8.0 Hz, H-9), 7.15 (dd, 1H, J 8.0, 1.2 Hz, H-8), 4.01 (s, 3H, O-C$\underline{H}_3$); δ_c (100 MHz, $CDCl_3$) 159.6 (C=O), 153.5 (q, $^2J_{CF}$ 35.5 Hz, C-2), 152.5 (C-10b), 147.8 (C-7), 142.8 (C-6a), 140.7 (C-4), 125.2 (C-9), 121.0 (q, $^1J_{CF}$ 275.5 Hz, $\underline{C}F_3$), 120.1 (q, $^3J_{CF}$ 2.2 Hz, C-3), 119.4 (C-10a), 119.3 (C-4a), 116.6 (C-10), 115.1 (C-8); δ_F (376 MHz, $CDCl_3$) -68.4 (s); *m/z* (ESI) 296 $[M+H]^+$ (Found: $[M+H]^+$, 296.0529. $C_{14}H_8NO_3F_3$ requires $[M+H]^+$, 296.0529).

3-Methylpyrido[3,2-*c*]coumarin

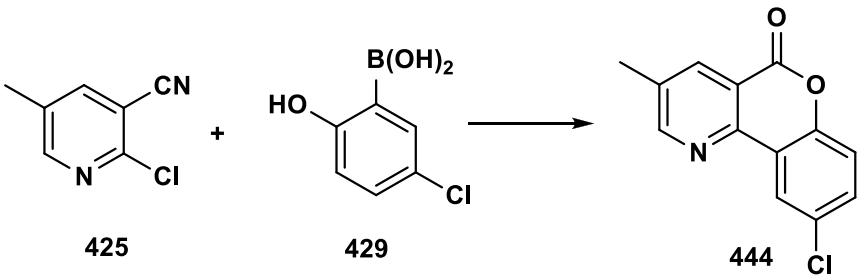


425 155 443

A mixture of 2-chloro5-methylnicotinonitrile (84 mg, 0.55 mmol, 1.0 equiv.), 2-hydroxyphenylboronic acid (90.7 mg, 0.66 mmol, 1.2 equiv.), tetrakis(triphenylphosphine)palladium(0) (63 mg, 0.05 mmol, 0.1 equiv.) and 1,4-dioxane (5.5 mL) was stirred at 80 ºC for 1 h, then water (0.55 mL) and potassium carbonate solution (2 M aqueous solution; 1.10 mL, 2.20 mmol, 4.0 equiv.) were added at rt and the resulting mixture stirred at 80 ºC for 12 h. The mixture was diluted with dichloromethane (150 mL) and water (20 mL). The layers were separated and the organic phase washed with brine (20 mL). The combined aqueous phases were extracted with dichloromethane (3 × 150 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (11.8% ethyl acetate-hexane) afforded 3-methylpyrido[3,2-*c*]coumarin (94 mg, 82%) as a white solid; R_f 0.65 (25%

ethyl acetate-hexane); mp 183-184 °C; ν_{max} (solid) 2926, 1717 (C=O), 1605, 1456, 1174, 754, 738 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.84 (d, 1H, J 2.2 Hz, H-2), 8.54 (dd, 1H, J 7.8, 1.7 Hz, H-10), 8.41 (d, 1H, J 2.2 Hz, H-4), 7.57-7.53 (m, 1H, H-8), 7.42-7.35 (m, 2H, H-7, H-9), 2.51 (s, 3H, 3-C<u>H</u>$_3$); δ_c (100 MHz, $CDCl_3$) 161.6 (C=O), 156.8 (C-2), 152.4 (C-6a), 149.6 (C-10b), 137.8 (C-4), 134.1 (C-3), 131.8 (C-8a), 125.0 (C-10), 124.5 (C-9), 119.5 (C-10a), 117.3 (C-7), 117.0 (C-4a), 18.6 (3-<u>C</u>H_3); *m/z* (ESI) 212 $[M+H]^+$ (Found: $[M+H]^+$, 212.0706. $C_{13}H_9NO_2$ requires $[M+H]^+$, 212.0706).

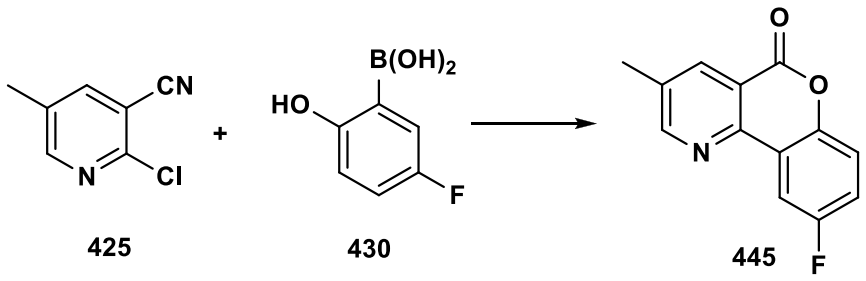
9-Chloro-3-methylpyrido[3,2-*c*]coumarin



A mixture of 2-chloro-5-methylnicotinonitrile (81 mg, 0.53 mmol, 1.0 equiv.), 5-chloro-2-hydroxyphenylboronic acid (100 mg, 0.64 mmol, 1.2 equiv.), tetrakis(triphenylphosphine)palladium(0) (62 mg, 0.05 mmol, 0.1 equiv.) and 1,4-dioxane (5.3 mL) was stirred at 80 ºC for 1 h, then water (0.53 mL) and potassium carbonate solution (2 M aqueous solution; 1.07 mL, 2.13 mmol, 4.0 equiv.) were added at rt and the resulting mixture stirred at 80 ºC for 12 h. The mixture was diluted with dichloromethane (150 mL) and water (20 mL). The layers were separated and the organic phase washed with brine (20 mL). The combined aqueous phases were extracted with dichloromethane (3 × 150 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (10% ethyl acetate-hexane) afforded 9-chloro-3-methylpyrido[3,2-*c*]coumarin (92 mg, 70%) as a white solid; R_f 0.74 (25% ethyl acetate-hexane); mp 197-198 °C; ν_{max} (solid) 3060, 1749 (C=O), 1621,

1402, 1243, 1109, 819, 715 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.86 (d, 1H, J 2.3 Hz, H-10), 8.54 (d, 1H, J 2.5 Hz, H-2), 8.42 (d, 1H, J 2.5 Hz, H-4), 7.50 (dd, 1H, J 8.8, 2.3 Hz, H-8), 7.32 (d, 1H, J 8.8 Hz, H-7), 2.53 (s, 3H, 3-C<u>H</u>$_3$); δ_c (100 MHz, $CDCl_3$) 161.0 (C=O), 157.0 (C-2), 150.8 (C-10b), 148.6 (C-6a), 137.9 (C-4), 134.9 (C-9), 131.8 (C-8), 130.7 (C-3), 124.3 (C-10), 120.9 (C-10a), 118.8 (C-7), 117.2 (C-4a), 18.7 (3-<u>C</u>H_3); *m/z* (ESI) 246 $[M+H]^+$ (Found: $[M+H]^+$, 246.0314. $C_{13}H_8NO_2Cl$ requires $[M+H]^+$, 246.0316).

9-Fluoro-3-methylpyrido[3,2-*c*]coumarin

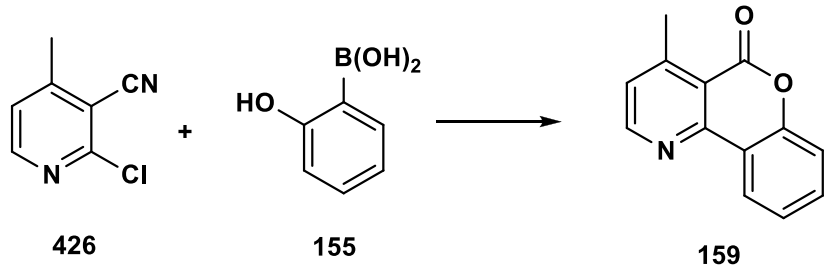


425 430 445

A mixture of 2-chloro-5-methylnicotinonitrile (84 mg, 0.55 mmol, 1.0 equiv.), 5-fluoro-2-hydroxyphenylboronic acid (91 mg, 0.66 mmol, 1.2 equiv.), tetrakis(triphenylphosphine)palladium(0) (64 mg, 0.06 mmol, 0.1 equiv.) and 1,4-dioxane (5.5 mL) was stirred at 80 ºC for 1 h, then water (0.55 mL) and potassium carbonate solution (2 M aqueous solution; 1.10 mL, 2.20 mmol, 4.0 equiv.) were added at rt and the resulting mixture stirred at 80 ºC for 12 h. The mixture was diluted with dichloromethane (150 mL) and water (20 mL). The layers were separated and the organic phase washed with brine (20 mL). The combined aqueous phases were extracted with dichloromethane (3 × 150 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (11.1% ethyl acetate-hexane) afforded 9-fluoro-3-methylpyrido[3,2-*c*]coumarin (94 mg, 74%) as a white solid; R_f

0.69 (25% ethyl acetate-hexane); mp 173-175°C; ν_{max} (solid) 3054, 1732 (C=O), 1562, 1466, 1251, 1160, 1078, 874, 823, 797, 722 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.85 (d, 1H, J 2.2 Hz, H-2), 8.41 (d, 1H, J 2.2 Hz, H-4), 8.20 (dd, 1H, J 8.7, 3.1 Hz, H-10), 7.35 (dd, 1H, J 9.0, 4.4 Hz, H-7), 7.27-7.22 (m, 1H, H-8), 2.53 (s, 3H, 3-C<u>H</u>$_3$); δ_c (100 MHz, $CDCl_3$) 161.2 (C=O), 159.6 (d, $^1J_{CF}$ 244.0 Hz, C-9), 156.9 (C-2), 148.8 (d, $^4J_{CF}$ 2.8 Hz, C-10b), 148.4 (d, $^4J_{CF}$ 2.1 Hz, C-6a), 137.9 (C-4), 134.9 (C-3), 120.9 (d, $^3J_{CF}$ 8.8 Hz, C-10a), 119.1 (d, $^2J_{CF}$ 24.8 Hz, C-8), 118.9 (d, $^3J_{CF}$ 8.3 Hz, C-7), 117.1 (C-4a), 110.4 ($^2J_{CF}$ 25.4 Hz, C-10), 18.7 (3-<u>C</u>H$_3$); δ_F (376 MHz, $CDCl_3$) -116.6 (s); *m/z* (ESI) 230 $[M+H]^+$ (Found: $[M+H]^+$, 230.0610. $C_{13}H_8NO_2F$ requires $[M+H]^+$, 230.0612).

4-Methylpyrido[3,2-*c*]coumarin

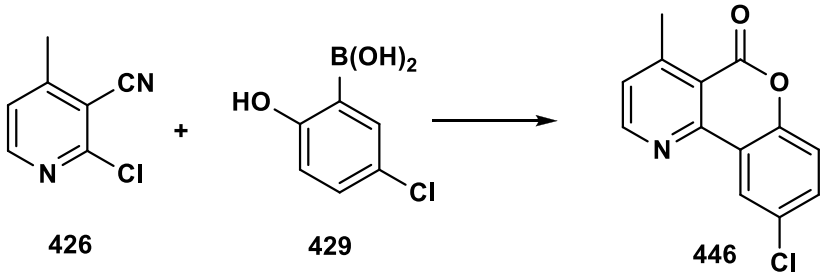


426 **155** **159**

A mixture of 2-chloro-4-methylnicotinonitrile (88 mg, 0.58 mmol, 1.0 equiv.), 2-hydroxyphenylboronic acid (96 mg, 0.69 mmol, 1.2 equiv.), tetrakis(triphenylphosphine)palladium(0) (67 mg, 0.06 mmol, 0.1 equiv.) and 1,4-dioxane (5.8 mL) was stirred at 80 ºC for 1 h, then water (0.58 mL) and potassium carbonate solution (2 M aqueous solution; 1.16 mL, 2.31 mmol, 4.0 equiv.) were added at rt and the resulting mixture stirred at 80 ºC for 12 h. The mixture was diluted with dichloromethane (150 mL) and water (20 mL). The layers were separated and the organic phase washed with brine (20 mL). The combined aqueous phases were extracted with dichloromethane (3 × 150 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (10.5% ethyl acetate-hexane) afforded 4-methylpyrido[3,2-*c*]coumarin (93 mg, 76%) as a white solid; R_f 0.58 (25%

ethyl acetate-hexane); mp 156-158 °C; ν_{max} (solid) 2957, 1731 (C=O), 1580, 1506, 1472, 762 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.74 (d, 1H, J 4.9 Hz, H-2), 8.53 (dd, 1H, J 7.4, 1.2 Hz, H-10), 7.50 (td, 1H, J 7.4, 1.2 Hz, H-8), 7.33-7.27 (m, 2H, H-7, H-9), 7.24 (d, 1H, J 4.9 Hz, H-3), 2.84 (s, 3H, 4-C<u>H</u>$_3$); δ_c (100 MHz, $CDCl_3$) 160.7 (C=O), 154.4 (C-2), 153.7 (C-6a), 153.1 (C-10b), 152.5 (C-4), 132.2 (C-8), 126.9 (C-10), 125.4 (C-9), 124.8 (C-3), 119.7 (C-10a), 116.9 (C-7), 116.7 (C-4a), 23.1 (4-<u>C</u>H_3); *m/z* (ESI) 212 $[M+H]^+$ (Found: $[M+H]^+$, 212.0704. $C_{13}H_9NO_2$ requires $[M+H]^+$, 212.0712). *In agreement with published data*.[132]

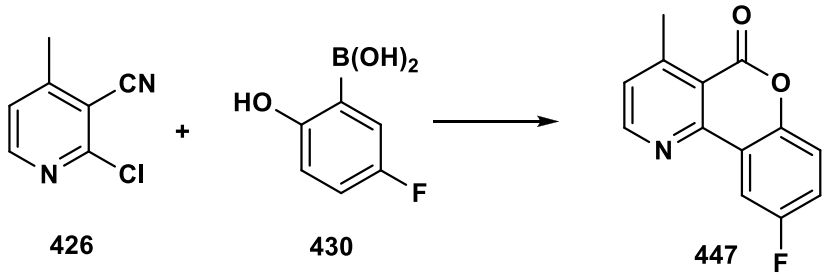
9-Chloro-4-methylpyrido[3,2-*c*]coumarin



A mixture of 2-chloro-4-methylnicotinonitrile (87 mg, 0.57 mmol, 1.0 equiv.), 5-chloro-2-hydroxyphenylboronic acid (118 mg, 0.68 mmol, 1.2 equiv.), tetrakis(triphenylphosphine)palladium(0) (66 mg, 0.06 mmol, 0.1 equiv.) and 1,4-dioxane (5.7 mL) was stirred at 80 ºC for 1 h, then water (0.57 mL) and potassium carbonate solution (2 M aqueous solution; 1.14 mL, 2.28 mmol, 4.0 equiv.) were added at rt and the resulting mixture stirred at 80 ºC for 12 h. The mixture was diluted with dichloromethane (150 mL) and water (20 mL). The layers were separated and the organic phase washed with brine (20 mL). The combined aqueous phases were extracted with dichloromethane (3 × 150 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (10% ethyl acetate-hexane) afforded 9-chloro-4-methylpyrido[3,2-*c*]coumarin (96 mg, 69%) as a white solid; R_f 0.69 (25% ethyl acetate-hexane); mp 190-192 °C; ν_{max} (solid) 3051, 1744 (C=O), 1658,

1572, 1435, 1241, 1114, 811, 692 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.80 (d, 1H, J 4.9 Hz, H-2), 8.58 (d, 1H, J 2.6 Hz, H-10), 7.50 (dd, 1H, J 8.7, 2.6 Hz, H-8), 7.34 (d, 1H, J 4.9 Hz, H-3), 7.29 (d, 1H, J 8.7 Hz, H-7), 2.88 (s, 3H, 4-C<u>H</u>$_3$); δ_c (100 MHz, $CDCl_3$) 160.1 (C=O), 154.5 (C-2), 153.8 (C-10b), 152.1 (C-6a), 151.0 (C-4), 132.2 (C-8), 130.5 (C-9), 127.4 (C-10), 125.2 (C-3), 121.0 (C-10a), 118.4 (C-7), 116.8 (C-4a), 23.0 (4-<u>C</u>H_3); *m/z* (ESI) 246 $[M+H]^+$ (Found: $[M+H]^+$, 246.0314. $C_{13}H_8NO_2Cl$ requires $[M+H]^+$, 246.0322).

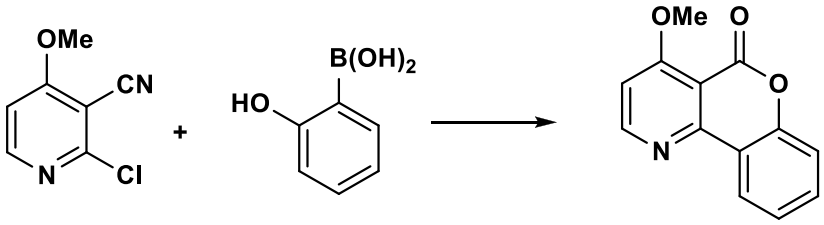
9-Fluoro-4-methylpyrido[3,2-*c*]coumarin



A mixture of 2-chloro-4-methylnicotinonitrile (91 mg, 0.60 mmol, 1.0 equiv.), 5-fluoro-2-hydroxyphenylboronic acid (112 mg, 0.72 mmol, 1.2 equiv.), tetrakis(triphenylphosphine)palladium(0) (69 mg, 0.06 mmol, 0.1 equiv.) and 1,4-dioxane (6.0 mL) was stirred at 80 ºC for 1 h, then water (0.30 mL) and potassium carbonate solution (2 M aqueous solution; 1.20 mL, 2.40 mmol, 4.0 equiv.) were added at rt and the resulting mixture stirred at 80 ºC for 12 h. The mixture was diluted with dichloromethane (150 mL) and water (20 mL). The layers were separated and the organic phase washed with brine (20 mL). The combined aqueous phases were extracted with dichloromethane (3 × 150 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (10.5 % ethyl acetate-hexane) afforded 9-fluoro-4-methylpyrido[3,2-*c*]coumarin (86 mg, 62%) as a white solid; R_f 0.62(25% ethyl acetate-hexane); mp 180-182 °C; ν_{max} (solid) 3084, 1735 (C=O), 1578, 1466, 1241, 1111, 1175, 1042, 891, 840, 813 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.81 (d, 1H, J

4.9 Hz, H-2), 8.27 (dd, 1H, J 8.9, 2.9 Hz, H-10), 7.35-7.31 (m, 2H, H-7, H-3), 7.29-7.24 (m, 1H, H-8), 2.89 (s, 3H, 4-C$\underline{H}_3$); δ_c (100 MHz, $CDCl_3$) 160.3 (C=O), 159.5 (d, $^1J_{CF}$ 243.4 Hz, C-9), 154.4 (C-2), 153.8 (C-4), 152.3 (d, $^4J_{CF}$ 2.8 Hz, C-10b), 148.6 (d, $^4J_{CF}$ 1.9 Hz, C-6a), 127.4 (C-3), 121.0 (d, $^3J_{CF}$ 8.7 Hz, C-10a), 119.6 (d, $^2J_{CF}$ 24.8 Hz, C-8), 118.4 (d, $^3J_{CF}$ 8.3 Hz, C-7), 116.7 (C-4a), 111.2 (d, $^2J_{CF}$ 25.5 Hz, C-10), 23.1 (4-$\underline{C}H_3$); δ_F (376 MHz, $CDCl_3$) -117.1 (s); *m/z* (ESI) 230 $[M+H]^+$ (Found: $[M+H]^+$, 230.0612. $C_{13}H_8NO_2F$ requires $[M+H]^+$, 230.0617).

4-Methoxypyrido[3,2-*c*]coumarin

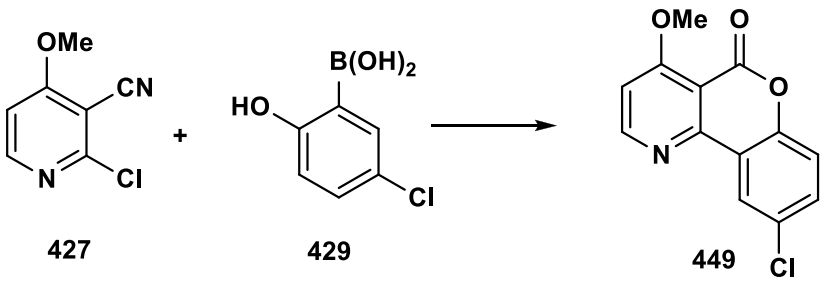


427 155 448

A mixture of 2-chloro-4-methoxynicotinonitrile (50.0 mg, 0.30 mmol, 1.0 equiv.), 2-hydroxyphenylboronic acid (49 mg, 0.36 mmol, 1.2 equiv.), tetrakis(triphenylphosphine)palladium(0) (34 mg, 0.03 mmol, 0.1 equiv.) and 1,4-dioxane (3.0 mL) was stirred at 80 ºC for 1 h, then water (0.30 mL) and potassium carbonate solution (2 M aqueous solution; 0.60 mL, 1.20 mmol, 4.0 equiv.) were added at rt and the resulting mixture stirred at 80 ºC for 12 h. The mixture was diluted with dichloromethane (150 mL) and water (20 mL). The layers were separated and the organic phase washed with brine (20 mL). The combined aqueous phases were extracted with dichloromethane (3 × 150 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (33.3% ethyl acetate-hexane) afforded 4-methoxypyrido[3,2-*c*]coumarin (48 mg, 72%) as a white solid; R_f 0.54 (80% ethyl acetate-hexane); mp 193-195 °C; ν_{max} (solid) 3092, 1729 (C=O), 1552, 1440, 1355, 1243, 1165, 1095, 1042, 762 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.81 (d, 1H, J 5.8 Hz,

H-2), 8.57 (dd, 1H, J 7.9, 1.2 Hz, H-10), 7.61-7.53 (m, 1H, H-8), 7.41-7.32 (m, 2H, H-7, H-9), 6.98 (d, 1H, J 5.8 Hz, H-3), 4.11 (s, 3H, O-C<u>H</u>$_3$); δ_c (100 MHz, $CDCl_3$) 168.1 (C-4), 158.1 (C=O), 156.4 (C-2), 154.9 (C-10b), 152.9 (C-6a), 132.5 (C-8), 125.6 (C-10), 124.6 (C-9), 119.3 (C-10a), 116.9 (C-7), 107.3 (C-4a), 106.6 (C-3), 56.9 (O-<u>C</u>H$_3$); *m/z* (ESI) 228 $[M+H]^+$ (Found: $[M+H]^+$, 228.0651. $C_{13}H_9NO_3$ requires $[M+H]^+$, 228.0661).

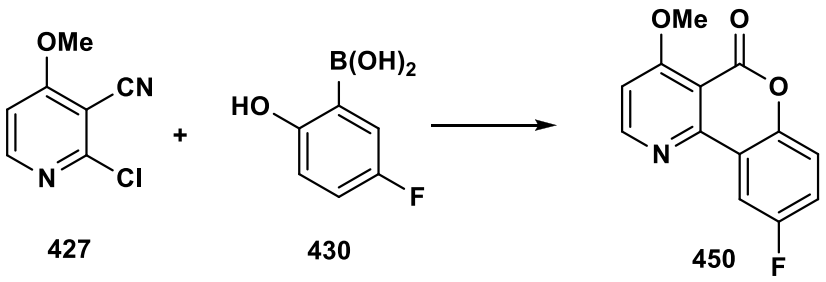
9-Chloro-4-methoxypyrido[3,2-*c*]coumarin



A mixture of 2-chloro-4-methoxynicotinonitrile (49 mg, 0.29 mmol, 1.0 equiv.), 5-chloro-2-hydroxyphenylboronic acid (60 mg, 0.35 mmol, 1.2 equiv.), tetrakis(triphenylphosphine)palladium(0) (34 mg, 0.03 mmol, 0.1 equiv.) and 1,4-dioxane (2.9 mL) was stirred at 80 ºC for 1 h, then water (0.29 mL) and potassium carbonate solution (2 M aqueous solution; 0.58 mL, 1.16 mmol, 4.0 equiv.) were added at rt and the resulting mixture stirred at 80 ºC for 12 h. The mixture was diluted with dichloromethane (150 mL) and water (20 mL). The layers were separated and the organic phase washed with brine (20 mL). The combined aqueous phases were extracted with dichloromethane (3 × 150 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (25% ethyl acetate-hexane) afforded 9-chloro-4-methoxypyrido[3,2-*c*]coumarin (51 mg, 66%) as a white solid; R_f 0.66 (80% ethyl acetate-hexane); mp 209-211 °C; ν_{max} (solid) 3087, 1749 (C=O),

1567, 1430, 1310, 1232, 1023, 839, 815 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.81 (d, 1H, J 5.8 Hz, H-2), 8.56 (d, 1H, J 2.6 Hz, H-10), 7.50 (dd, 1H, J 8.8, 2.6 Hz, H-8), 7.01 (d, 1H, J 5.8 Hz, H-3), 4.11 (s, 3H, O-C$\underline{H}_3$); δ_c (100 MHz, $CDCl_3$) 168.1 (C-4), 157.4 (C=O), 156.5 (C-2), 153.8 (C-10b), 151.3 (C-6a), 132.4 (C-8), 130.3 (C-9), 125.3 (C-10), 120.5 (C-10a), 118.4 (C-7), 107.1 (C-3), 106.0 (C-4a), 57.0 (O-$\underline{C}H_3$); *m/z* (ESI) 262 $[M+H]^+$ (Found: $[M+H]^+$, 262.0266. $C_{13}H_8NO_3Cl$ requires $[M+H]^+$, 262.0271).

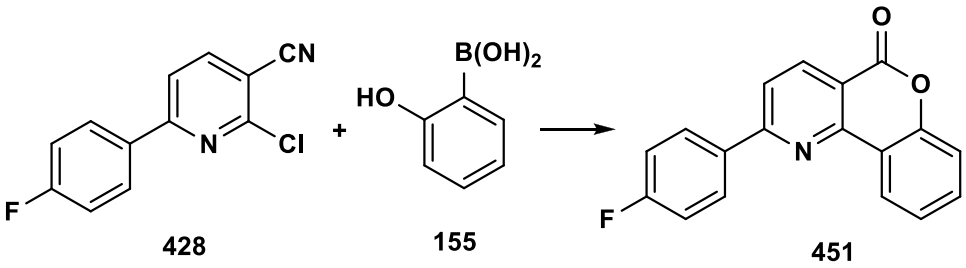
9-Fluoro-4-methoxypyrido[3,2-*c*]coumarin



A mixture of 2-chloro-4-methoxynicotinonitrile (52 mg, 0.31 mmol, 1.0 equiv.), 5-fluoro-2-hydroxyphenylboronic acid (58 mg, 0.37 mmol, 1.2 equiv.), tetrakis(triphenylphosphine)palladium(0) (36 mg, 0.03 mmol, 0.1 equiv.) and 1,4-dioxane (3.1 mL) was stirred at 80 ºC for 1 h, then water (0.31 mL) and potassium carbonate solution (2 M aqueous solution; 0.62 mL, 1.24 mmol, 4.0 equiv.) were added at rt and the resulting mixture stirred at 80 ºC for 12 h. The mixture was diluted with dichloromethane (150 mL) and water (20 mL). The layers were separated and the organic phase washed with brine (20 mL). The combined aqueous phases were extracted with dichloromethane (3 × 150 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (28.6% ethyl acetate-hexane) afforded 9-fluoro-4-methoxypyrido[3,2-*c*]coumarin (46 mg, 60%) as a white solid; R_f 0.82 (55.6% acetone-hexane); mp 204-206 °C; ν_{max} (solid) 3073, 1720, 1567, 1466, 1243, 1173, 1080, 1021, 891, 824 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.80 (d, 1H, J 5.8 Hz,

H-2), 8.22 (dd, 1H, J 8.9, 2.9 Hz, H-10), 7.33-7.27 (m, 2H, H-7, H-8), 7.00 (d, 1H, J 5.8 Hz, H-3), 4.11 (s, 3H, O-C<u>H</u>$_3$); δ_c (100 MHz, $CDCl_3$) 168.0 (C-4), 159.4 (d, $^1J_{CF}$ Hz, C-9), 157.8 (C=O), 156.5 (C-2), 154.0 (d, $^4J_{CF}$ 2.8 Hz, C-10b), 148.9 (d, $^4J_{CF}$ 1.9 Hz, C-6a), 120.5 (d, $^3J_{CF}$ 8.8 Hz, C-10a), 119.9 (d, $^2J_{CF}$ 24.8 Hz, C-8), 118.5 (d, $^3J_{CF}$ 8.2 Hz, C-7), 111.3 ($^2J_{CF}$ 25.4 Hz, C-10), 107.3 (C-4a), 107.1 (C-3), 57.0 (O-<u>C</u>H$_3$); δ_F (376 MHz, $CDCl_3$) -117.2 (s); *m/z* (ESI) 246 $[M+H]^+$ (Found: $[M+H]^+$, 246.0560. $C_{13}H_8NO_3F$ requires $[M+H]^+$, 246.0567).

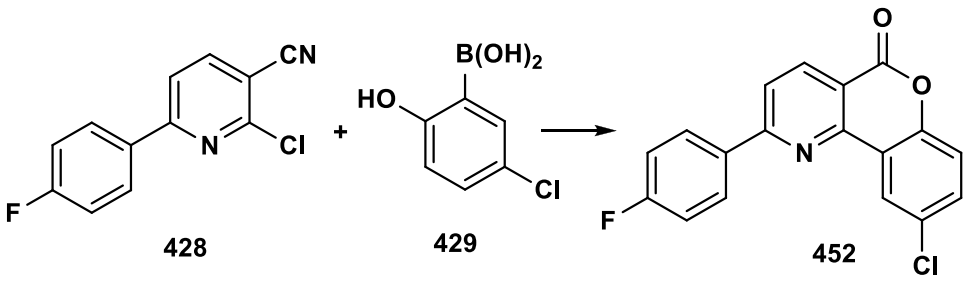
2-(4-Fluorophenyl)pyrido[3,2-*c*]coumarin



A mixture of 2-chloro-6-(4-fluorophenyl)nicotinonitrile (105 mg, 0.45 mmol, 1.0 equiv.), 2-hydroxyphenylboronic acid (75 mg, 0.54 mmol, 1.2 equiv.), tetrakis(triphenylphosphine)palladium(0) (52 mg, 0.05 mmol, 0.1 equiv.) and 1,4-dioxane (4.5 mL) was stirred at 80 ºC for 1 h, then water (0.45 mL) and potassium carbonate solution (2 M aqueous solution; 0.91 mL, 1.82 mmol, 4.0 equiv.) were added at rt and the resulting mixture stirred at 80 ºC for 12 h. The mixture was diluted with dichloromethane (250 mL) and water (20 mL). The layers were separated and the organic phase washed with brine (20 mL). The combined aqueous phases were extracted with dichloromethane (3 × 250 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (11.1% ethyl acetate-hexane) afforded 2-(4-fluorophenyl)pyrido[3,2-*c*]coumarin (106 mg, 80%) as a white solid; R_f 0.55 (22.2% ethyl acetate-hexane); mp 225-226 °C; ν_{max} (solid) 3077, 1727 (C=O), 1599, 1575, 1422, 1230, 1106, 829, 801, 752 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.74 (dd, 1H,

J 7.9, 1.6 Hz, H-10), 8.65 (d, 1H, J 8.4 Hz, H-3), 8.30-8.25 (m, 2H, H-2', H-6'), 7.92 (d, 1H, J 8.4 Hz, H-4), 7.61 (ddd, 1H, J 8.3, 7.4, 1.6 Hz, H-8), 7.46-7.40 (m, 2H, H-7, H-9), 7.30-7.22 (m, 2H, H-3', H-5'); δ_c (100 MHz, $CDCl_3$) 164.8 (d, $^1J_{CF}$ 251.8 Hz, C-4'), 161.6 (C-2), 161.3 (C=O), 153.1 (C-6a), 151.9 (C-10b), 139.3 (C-4), 134.2 (d, $^4J_{CF}$ 3.0 Hz, C-1'), 132.4 (C-8), 130.0 (C-10), 129.9 (C-9), 125.0 (d, $^3J_{CF}$ 8.4 Hz, C-2', C-6'), 120.1 (C-7), 119.7 (C-10a), 117.4 (C-3), 116.3 (d, $^2J_{CF}$ 21.8 Hz, C-3', C-5'), 115.7 (C-4a); δ_F (376 MHz, $CDCl_3$) -109.7 (s); *m/z* (ESI) 292 $[M+H]^+$ (Found: $[M+H]^+$, 292.0770. $C_{18}H_{10}NO_2F$ requires $[M+H]^+$, 292.0774).

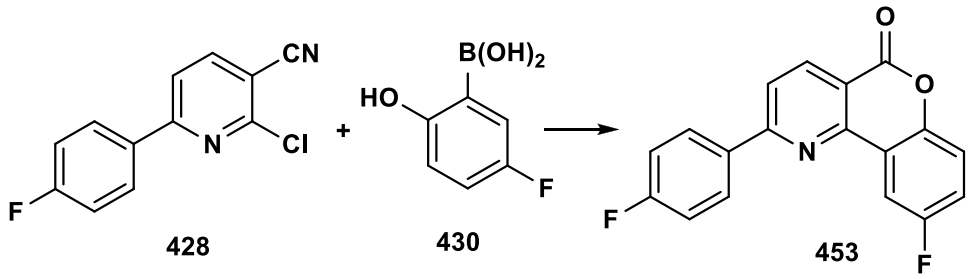
9-Chloro-2-(4-fluorophenyl)pyrido[3,2-*c*]coumarin



A mixture of 2-chloro-6-(4-fluorophenyl)nicotinonitrile (99 mg, 0.42 mmol, 1.0 equiv.), 5-chloro-2-hydroxyphenylboronic acid (79 mg, 0.51 mmol, 1.2 equiv.), tetrakis(triphenylphosphine)palladium(0) (49 mg, 0.04 mmol, 0.1 equiv.) and 1,4-dioxane (4.2 mL) was stirred at 80 ºC for 1 h, then water (0.42 mL) and potassium carbonate solution (2 M aqueous solution; 0.85 mL, 1.69 mmol, 4.0 equiv.) were added at rt and the resulting mixture stirred at 80 ºC for 12 h. The mixture was diluted with dichloromethane (250 mL) and water (20 mL). The layers were separated and the organic phase washed with brine (20 mL). The combined aqueous phases were extracted with dichloromethane (3 × 250 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (10.5% ethyl acetate-hexane) afforded 9-chloro-2-(4-fluorophenyl)pyrido[3,2-*c*]coumarin (103 mg, 75%) as a white solid; R_f 0.71 (29.4% ethyl acetate-hexane); mp 234-235 °C; ν_{max} (solid) 3097, 1740

(C=O), 1598, 1574, 1405, 1233, 1160, 833, 813, 797 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.69 (d, 1H, J 2.6 Hz, H-10), 8.66 (d, 1H, J 8.4 Hz, H-4), 8.30-8.25 (m, 2H, H-2', H-6'), 7.97 (d, 1H, J 8.4 Hz, H-4), 7.55 (dd, 1H, J 8.8, 2.6 Hz, H-8), 7.36 (d, 1H, J 8.8 Hz, H-7), 7.30-7.24 (m, 2H, H-3', H-5'); δ_c (100 MHz, $CDCl_3$) 164.8 (d, $^1J_{CF}$ 252.3 Hz, C-4'), 161.7 (C-2), 160.6 (C=O), 151.3 (C-10b), 150.7 (C-6a), 139.2 (C-4), 133.7 (d, $^4J_{CF}$ 3.1 Hz, C-1'), 132.2 (C-8), 130.5 (C-9), 129.9 (d, $^3J_{CF}$ 8.8 Hz, C-2', C-6'), 124.5 (C-10), 120.8 (C-10a), 120.6 (C-3), 118.8 (C-7), 116.2(d, $^2J_{CF}$ 21.9 Hz, C-3', C-5'), 115.7 (C-4a); δ_F (376 MHz, $CDCl_3$) -109.2 (s); *m/z* (ESI) 326 $[M+H]^+$ (Found: $[M+H]^+$, 326.0378. $C_{18}H_9NO_2FCl$ requires $[M+H]^+$, 326.0384).

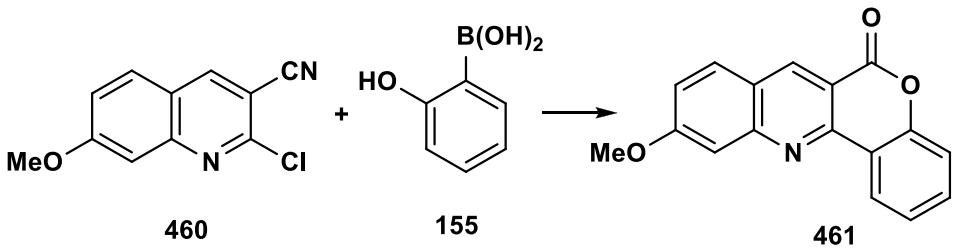
2-(4-Fluorophenyl)-9-fluoropyrido[3,2-*c*]coumarin



A mixture of 2-chloro-6-(4-fluorophenyl)nicotinonitrile (97 mg, 0.42 mmol, 1.0 equiv.), 5-fluoro-2-hydroxyphenylboronic acid (86 mg, 0.50 mmol, 1.2 equiv.), tetrakis(triphenylphosphine)palladium(0) (48 mg, 0.04 mmol, 0.1 equiv.) and 1,4-dioxane (4.2 mL) was stirred at 80 ºC for 1 h, then water (0.42 mL) and potassium carbonate solution (2 M aqueous solution; 0.83 mL, 1.66 mmol, 4.0 equiv.) were added at rt and the resulting mixture stirred at 80 ºC for 12 h. The mixture was diluted with dichloromethane (250 mL) and water (20 mL). The layers were separated and the organic phase washed with brine (20 mL). The combined aqueous phases were extracted with dichloromethane (3 × 250 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (11.1% ethyl acetate-hexane) afforded 2-(4-fluorophenyl)-9-fluoropyrido[3,2-*c*]coumarin (93 mg, 72%) as a white solid; R_f 0.61 (22.2% ethyl acetate-hexane); mp 224-226 °C; ν_{max} (solid) 3086, 1727

(C=O), 1579, 1567, 1470, 1231, 1157, 827, 799, 792 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.66 (d, 1H, J 8.4, H-3), 8.38 (dd, 1H, J 8.6, 3.0 Hz, H-10), 8.29-8.23 (m, 2H, H-2', H-6'), 7.96 (d, 1H, J 8.4 Hz, H-4), 7.39 (dd, 1H, J 9.0, 4.4 Hz, H-7), 7.34-7.30 (m, 1H, H-8), 7.29-7.23 (m, 2H, H-3', H-5'); δ_c (100 MHz, $CDCl_3$) 164.9 (d, $^1J_{CF}$ 252.1 Hz, C-4'), 161.7 (C-2), 160.9 (C=O), 159.6 (d, $^1J_{CF}$ 244.1 Hz, C-9), 151.1 (d, $^4J_{CF}$ 2.8 Hz, C-6a), 149.1 (d, $^4J_{CF}$ 2.1 Hz, C-10b), 139.3 (C-4), 133.9 (d, $^4J_{CF}$ 3.2 Hz, C-1'), 130.0 (d, $^3J_{CF}$ 8.7 Hz, C-2', C-6'), 121.0 (d, $^3J_{CF}$ 8.6 Hz, C-10a), 120.7 (C-3), 119.7 (d, $^2J_{CF}$ 27.8 Hz, C-8), 119.1 (d, $^3J_{CF}$ 8.3 Hz, C-7), 116.3 (d, $^2J_{CF}$ 21.8 Hz, C-3', C-5'), 115.8 (C-4a), 110.7 ($^2J_{CF}$ 25.2 Hz, C-10); δ_F (376 MHz, $CDCl_3$) -109.3 (s), -116.6 (s); *m/z* (ESI) 310 $[M+H]^+$ (Found: $[M+H]^+$, 310.0677. $C_{18}H_9NO_2F_2$ requires $[M+H]^+$, 310.0680).

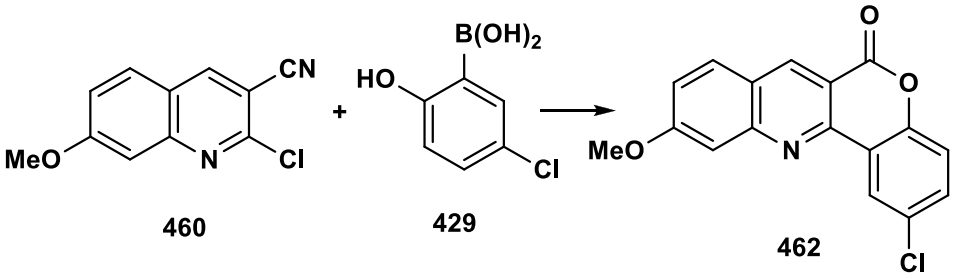
10-Methoxy-6*H*-chromeno[4,3-*b*]quinolin-6-one



A mixture of 2-chloro-7-methoxyquinoline-3-carbonitrile (97 mg, 0.45 mmol, 1.0 equiv.), 2-hydroxyphenylboronic acid (74 mg, 0.53 mmol, 1.2 equiv.), tetrakis(triphenylphosphine)palladium(0) (51 mg, 0.04 mmol, 0.1 equiv.) and 1,4-dioxane (4.5 mL) was stirred at 80 ºC for 1 h, then water (0.45 mL) and potassium carbonate solution (2 M aqueous solution; 0.89 mL, 1.78 mmol, 4.0 equiv.) were added at rt and the resulting mixture stirred at 80 ºC for 12 h. The mixture was diluted with dichloromethane (150 mL) and water (20 mL). The layers were separated and the organic phase washed with brine (20 mL). The combined aqueous phases were extracted with dichloromethane (3 × 150 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (11.1% ethyl acetate-hexane) afforded 10-methoxy-6*H*-chromeno[4,3-*b*]quinolin-6-one (92 mg, 75%) as a white solid; R_f 0.69 (20% ethyl acetate-hexane); mp 231-233°C; ν_{max} (solid) 2985, 1735

(C=O), 1603, 1482, 1242, 1177, 831, 728 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 9.11 (s, 1H, H-7), 8.76 (dd, 1H, J 7.9, 1.7 Hz, H-1), 7.89 (d, 1H, J 9.0 Hz, H-8), 7.59 (ddd, 1H, J 8.2, 7.4, 1.7 Hz, H-3), 7.52 (d, 1H, J 2.4 Hz, H-11), 7.46-7.37 (m, 2H, H-4, H-2), 7.28 (dd, 1H, J 9.0, 2.4 Hz, H-9), 4.05 (s, 3H, O-C<u>H</u>$_3$); δ_c (100 MHz, $CDCl_3$) 164.2 (C-10), 161.8 (C=O), 153.6 (C-4a), 153.0 (C-12a), 150.3 (C-11a), 140.4 (C-7), 132.4 (C-3), 130.7 (C-8), 125.3 (C-1), 125.0 (C-2), 123.2 (C-7a), 121.7 (C-12b), 119.9 (C-9), 117.6 (C-4), 113.9 (C-6a), 107.1 (C-11), 56.0 (O-<u>C</u>H_3); *m/z* (ESI) 278 $[M+H]^+$ (Found: $[M+H]^+$, 278.0812. $C_{17}H_{11}NO_3$ requires $[M+H]^+$, 228.0817).

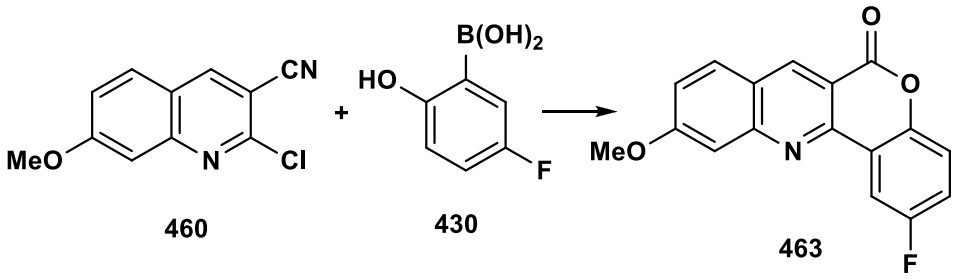
2-Chloro-10-methoxy-6*H*-chromeno[4,3-*b*]quinolin-6-one



A mixture of 2-chloro-7-methoxyquinoline-3-carbonitrile (99 mg, 0.45 mmol, 1.0 equiv.), 5-chloro-2-hydroxyphenylboronic acid (94 mg, 0.54 mmol, 1.2 equiv.), tetrakis(triphenylphosphine)palladium(0) (52 mg, 0.05 mmol, 0.1 equiv.) and 1,4-dioxane (4.5 mL) was stirred at 80 ºC for 1 h, then water (0.45 mL) and potassium carbonate solution (2 M aqueous solution; 0.91 mL, 1.81 mmol, 4.0 equiv.) were added at rt and the resulting mixture stirred at 80 ºC for 12 h. The mixture was diluted with dichloromethane (150 mL) and water (20 mL). The layers were separated and the organic phase washed with brine (20 mL). The combined aqueous phases were extracted with dichloromethane (3 × 150 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (10% ethyl acetate-hexane) afforded 2-chloro-10-methoxy-6*H*-chromeno[4,3-*b*]quinolin-6-one (94 mg, 67%) as a

white solid; R_f 0.78 (20% ethyl acetate-hexane); mp 241-242 °C; ν_{max} (solid) 2997, 1738 (C=O), 1607, 1473, 1231, 1172, 1015, 821, 724 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 9.10 (s, 1H, H-7), 8.73 (d, 1H, J 2.5 Hz, H-1), 7.90 (d, 1H, J 9.0 Hz, H-8), 7.54-7.51 (m, 2H, H-11, H-3), 7.35-7.29 (m, 2H, H-9, H-4), 4.06 (s, 3H, O-C<u>H</u>$_3$); δ_c (100 MHz, $CDCl_3$) 164.4(C-10), 161.3 (C=O), 153.5 (C-4a), 151.3 (C-12a), 149.1 (C-11a), 140.5 (C-7), 132.2 (C-3), 130.7 (C-8), 130.6 (C-2), 124.8 (C-1), 123.3 (C-7a), 122.1 (C-9), 121.2 (C-12b), 119.0 (C-4), 113.6 (C-6a), 107.0 (C-11), 56.1 (O-<u>C</u>H_3); *m/z* (ESI) 312 $[M+H]^+$ (Found: $[M+H]^+$, 312.0424. $C_{17}H_{10}NO_3Cl$ requires $[M+H]^+$, 312.0428).

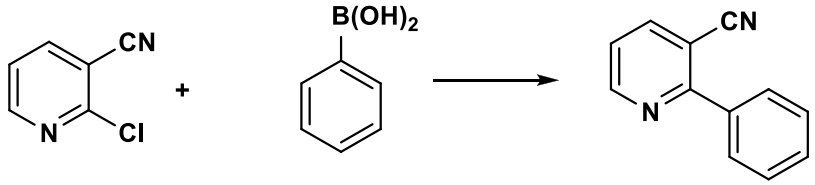
2-Fluoro-10-methoxy-6*H*-chromeno[4,3-*b*]quinolin-6-one



A mixture of 2-chloro-7-methoxyquinoline-3-carbonitrile (104 mg, 0.47 mmol, 1.0 equiv.), 5-fluoro-2-hydroxyphenylboronic acid (89 mg, 0.57 mmol, 1.2 equiv.), tetrakis(triphenylphosphine)palladium(0) (55 mg, 0.05 mmol, 0.1 equiv.) and 1,4-dioxane (4.7 mL) was stirred at 80 ºC for 1 h, then water (0.47 mL) and potassium carbonate solution (2 M aqueous solution; 0.95 mL, 1.90 mmol, 4.0 equiv.) were added at rt and the resulting mixture stirred at 80 ºC for 12 h. The mixture was diluted with dichloromethane (150 mL) and water (20 mL). The layers were separated and the organic phase washed with brine (20 mL). The combined aqueous phases were extracted with dichloromethane (3 × 150 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (10.5% ethyl acetate-hexane) afforded 2-fluoro-10-methoxy-6*H*-chromeno[4,3-*b*]quinolin-6-one (87 mg, 62%) as a white solid; R_f 0.75 (20% ethyl acetate-hexane); mp 222-224 °C; ν_{max} 2954, 1740

(C=O), 1467, 1228, 1161, 1119, 1006, 815, 720 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 9.12 (s, 1H, H-7), 8.43 (dd, 1H, J 8.8, 3.1 Hz, H-1), 7.91 (d, 1H, J 9.0 Hz, H-8), 7.53 (d, 1H, J 2.4 Hz, H-11), 7.38 (dd, 1H, J 9.0, 2.4 Hz, H-9), 7.33-7.29 (m, 2H, H-3, H-4), 4.06 (s, 3H, O-C$\underline{H}_3$); δ_c (100 MHz, $CDCl_3$) 164.4 (C-10), 161.4 (C=O), 159.7 (d, $^1J_{CF}$ 243.9 Hz, C-2), 153.5 (C-12a), (d, $^4J_{CF}$ 2.7 Hz, C-4a), 149.1 (C-11a), 140.4 (C-7), 130.7 (C-8), 123.4 (C-7a), 122.1 (C-9), 121.3 (d, $^3J_{CF}$ 8.4 Hz, C-12b), 119.6 ($^2J_{CF}$ 24.6 Hz, C-3), 119.2 (d, $^3J_{CF}$ 8.3 Hz, C-4), 113.6 (C-6a), 111.0 ($^2J_{CF}$ 25.2 Hz, C-1), 107.1 (C-11), 56.1 (O-$\underline{C}H_3$); δ_F (376 MHz, $CDCl_3$) -117.0 (s); *m/z* (ESI) 296 $[M+H]^+$ (Found: $[M+H]^+$, 296.0719. $C_{17}H_{10}NO_3F$ requires $[M+H]^+$, 296.0723).

2-Phenylnicotinonitrile



163 **148** **470**

A mixture of 2-chloronicotinonitrile (115 mg, 0.83 mmol, 1.0 equiv.), phenylboronic acid (122 mg, 1.00 mmol, 1.2 equiv.), tetrakis(triphenylphosphine)palladium(0) (96 mg, 0.08 mmol, 0.1 equiv.) and 1,4-dioxane (8.3 mL) was stirred at 80 ºC for 1 h, then water (0.83 mL) and potassium carbonate solution (2 M aqueous solution; 1.67 mL, 3.33 mmol, 4.0 equiv.) were added at rt and the resulting mixture stirred at 80 ºC for 12 h. The mixture was diluted with dichloromethane (150 mL) and water (20 mL). The layers were separated and the organic phase washed with brine (20 mL). The combined aqueous phases were extracted with dichloromethane (3 × 150 mL) and the combined organic phases dried (Na_2SO_4). Concentration under reduced pressure and chromatography (20% ethyl acetate-hexane) afforded 2-phenylnicotinonitrile (142 mg, 94%) as a white solid; R_f 0.38 (20% ethyl acetate-hexane); mp 94-95 °C; ν_{max} (solid) 3069, 2224 (CN), 1578, 1552, 1430, 806, 740, 691 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.88 (dd, 1H, J 4.8, 1.8 Hz, H-6), 8.08 (dd, 1H, J 7.9, 1.8 Hz, H-6), 7.94-7.92 (m, 2H, H-2', H-6'), 7.56-7.51 (3H, m, H-3', H-5', H-4'), 7.38 (dd, 1H, J 7.9, 4.8 Hz, H-5); δ_c (100 MHz,

$CDCl_3$) 161.2 (C-2), 152.8 (C-6), 142.0 (C-4), 137.3 (C-1'), 130.4 (C-4'), 129.0 (C-2', C-6'), 128.8 (C-3', C-5'), 121.7 (C-5), 117.8 (CN), 107.6 (C-3); *m/z* (ESI) 181 $[M+H]^+$ (Found: $[M+H]^+$, 181.0758. $C_{12}H_8N_2$ requires $[M+H]^+$, 181.0760). *In agreement with published data*.[151]

3.3 *In Vitro* β-Haematin Inhibition Assay

Method I:

Nonidet P-40 mediated β-haematin formation was performed by the method previously described.[110] A 25 mM haemin stock solution was prepared by dissolving haemin in DMSO and filtering through a 0.22 μm polyvinylidene difluoride (PVDF) membrane syringe driven filter unit. Haemin stock (177.8 μL) was suspended in 2.0 M sodium acetate-acetic acid buffer solution (pH 4.9, 20 mL), resulting in a 222.2 μM homogeneous haematin suspension.

Deionised H_2O (80 μL) and NP-40 (305.5 μM; 20 μL) were added to each well, followed by the delivery of test sample solutions in DMSO (10 μL) at different concentrations in triplicate. The homogeneous haematin suspension (90 μL) was added to each well finally. The plate was covered and incubated at 37 °C for 4 h in a water bath and shaken at 55 rpm.

The plate was removed from incubation and centrifuged at 1100 × g for 1 h at 25°C in a Beckman Coulter Allegra 25R rotor. The supernatant was discarded, and then sodium bicarbonate solution (0.15 M; 200 μL) and SDS solution (2 % (w/v)) were added to each well. Centrifugation was repeated, and the supernatant was removed again. A final

addition of sodium hydroxide solution (0.36 M; 200 μL) and SDS solution (2 % (w/v)) was added to dissolve the β-haematin produced. The absorbance of the resulting mixture was measured at 400 nm on a BMG Labtech POLARstar Optima multiplate reader.

Method II:

Nonidet P-40 mediated β-haematin formation was performed by the colorimetric pyridine-based protocol previously described.[108] A 25 mM haemin stock solution was prepared by dissolving haemin in DMSO and filtering through a 0.22 μm polyvinylidene difluoride (PVDF) membrane syringe driven filter unit. Haemin stock (177.8 μL) was suspended in 2.0 M sodium acetate-acetic acid buffer solution (pH 4.9, 20 mL), resulting in a 222.2 μM homogeneous haematin suspension.

Deionised H_2O (73 μL), NP-40 (305.5 μM; 20 μL) and acetone (7 μL) were added to each well, followed by the delivery of test sample solutions in DMSO (10 μL) at different concentrations in triplicate. The homogeneous haematin suspension (90 μL) was added to each well finally. The plate was covered and incubated at 37 °C for 4 h in a water bath and shaken at 45 rpm.

The plate was removed from incubation after 4 h. 22.2 μL of the solution of 50% pyridine, 20% acetone, 20% water and 10% 200 mM HEPES buffer (pH 7.4) was added to each well, so that the final concentration of pyridine was 5% (v/v). Following the 10 min of shaking, the absorbance of the resulting complex was measured at 405 nm on a BMG Labtech POLARstar Optima multiplate reader. Sigmoidal concentration-response curves and the IC_{50} values were acquired from the software of GraphPad Prism v5.0.

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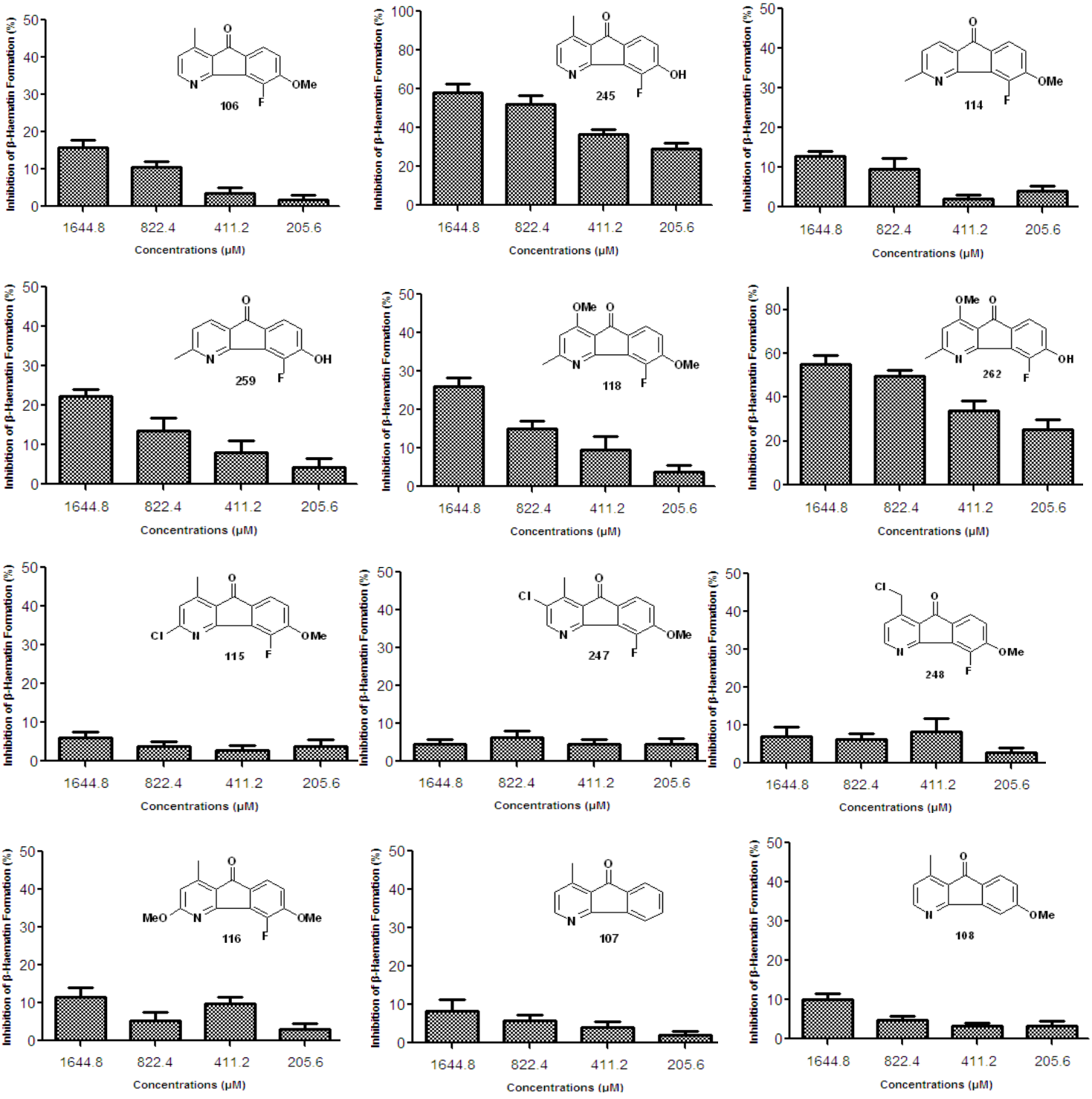
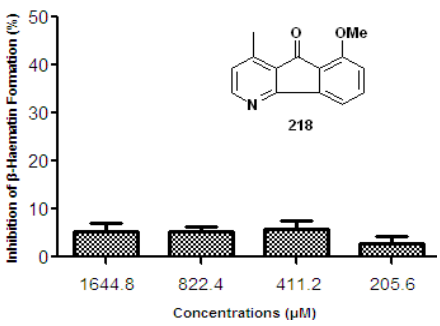
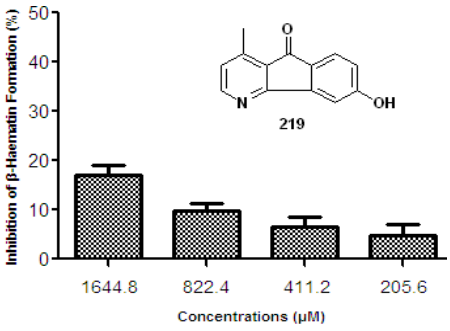
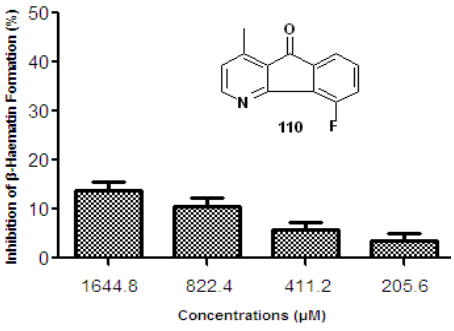
APPENDICES

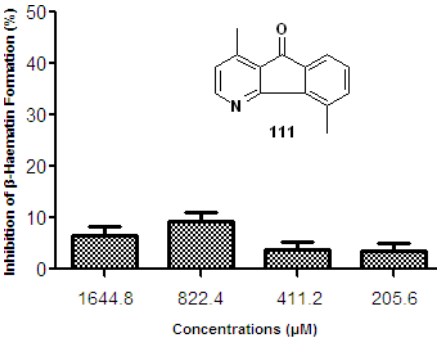
Appendix I Inhibitory activity of azafluorenones against β-haematin formation.

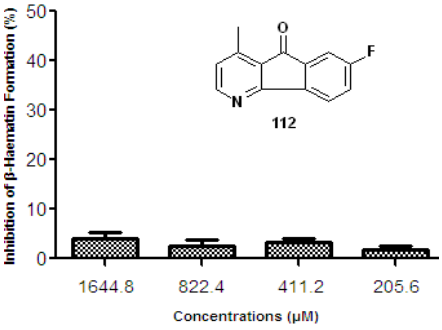


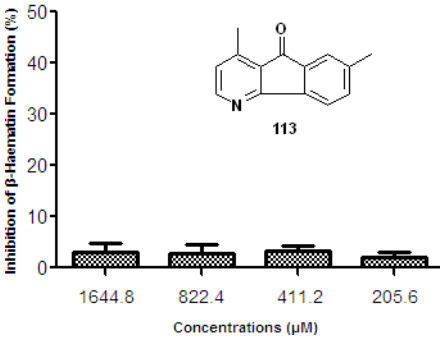

Inhibition of β-Haematin Formation (%)
50
40
30
20
10
0
O
OMe
N
218
1644.8
822.4
411.2
205.6
Concentrations (μM)


Inhibition of β-Haematin Formation (%)
50
40
30
20
10
0
O
OH
N
219
1644.8
822.4
411.2
205.6
Concentrations (μM)


Inhibition of β-Haematin Formation (%)
50
40
30
20
10
0
O
F
N
110
1644.8
822.4
411.2
205.6
Concentrations (μM)


Inhibition of β-Haematin Formation (%)
50
40
30
20
10
0
O
N
111
1644.8
822.4
411.2
205.6
Concentrations (μM)


Inhibition of β-Haematin Formation (%)
50
40
30
20
10
0
O
F
N
112
1644.8
822.4
411.2
205.6
Concentrations (μM)


Inhibition of β-Haematin Formation (%)
50
40
30
20
10
0
O
N
113
1644.8
822.4
411.2
205.6
Concentrations (μM)

Appendix II Inhibitory activity of azabiaryls against β-haematin formation.